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Page 44, line 2, "simple apparatus" should read "simple digestive apparatus."

Page 221, Table V, 7th column, line 1 under "Per cent." should read "0.300." Last column, line 1, under "Atmospheres" should read "4.459."

Page 265, line 14, "not noticeably sunken" should read "noticeably sunken."

Page 271, line 11 from bottom, "Hedivigia" should read "Hedwigia."

Page 491, line 3, "Table II" should read "Table V."

Page 517, footnote under Table III, line 3 after "ninth day" omit "the." Line 4, omit "the" before "two."

Page 652, line 20, "*P. graminis tritici compacti* under greenhouse conditions." should read "*P. graminis tritici*."

BUREAU OF ECONOMIC BOTANY, GOVERNMENT BOTANICAL GARDENS, LAL-BAGH, BANGALORE.
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STUDIES ON CAPACITIES OF SOILS FOR IRRIGATION WATER, AND ON A NEW METHOD OF DETERMINING VOLUME WEIGHT

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INTRODUCTION

In connection with a study of the economical duty of water for alfalfa in Sacramento Valley, California, conducted from 1910 to 1915 as a part of the Cooperative Irrigation Investigations in California [1] certain observations were made, and methods devised which it seemed could be better presented and described in a separate paper than as a part of the general report of the study. It is realized that some of the data presented suggest ideas concerning soil properties which are not fully established by the preliminary investigations here reported. The observations are presented in two parts: (1) Studies on the capacities of soils for irrigation water, and (2) a new method of determining the volume weight of soils.

PART I.—STUDIES ON CAPACITIES OF SOILS TO RETAIN IRRIGATION WATER [2]

THEORETICAL DISCUSSION

That soil water is held in the form of minute films about the soil particles and in the interstitial spaces is a matter of common knowledge. The maximum capacity of soils to hold water in the capillary form may be limited by the total interstitial space in the soil rather than by the total external surface area of the soil particles (*4*).[3] It is therefore obvious that the volume weight of soil in place may be an important indicator of its water-holding power. The various laboratory methods which have been used to determine the maximum retentive power of soils for water usually give results which are far in excess of the retentive

[1] The Cooperative Irrigation Investigations in California are carried on by the Division of Irrigation Investigations, Office of Public Roads and Rural Engineering, United States Department of Agriculture, in conjunction with the California State Department of Engineering and the University of California Agricultural Experiment Station.

[2] For a full report of general studies referred to herein, see (*1*) in "Literature cited," p. 34.

[3] Reference is made by number (italic) to "Literature cited," p. 34-35.

powers of the same soils under field conditions because, first, they consider a very short column of soil which is acted upon by special capillary forces; and, second, the samples of soil used have in most cases volume weights which are much lower than those obtaining in the undisturbed condition. It is desirable, especially where irrigation is practiced, to have accurate knowledge of the maximum water-holding capacity of the soil in place. Burr (5) found the maximum capacity of a fine, sandy loam (loess) to be 16 to 18 per cent of the weight of the dry soil. Quantities of water found by various investigators after heavy irrigations or rainfall (*2, 7, 8, 9, 11, 12*) seem to be in agreement with the results of Burr's experiment. Indirectly, therefore, the maximum water capacities of soils in place have been determined by a number of workers under various conditions.

The optimum quantity of water to add to a given depth of soil in a single irrigation is dependent on the moisture content of the soil before irrigation and its maximum water capacity.

Let P = the percentage of water to be added;
W = the weight of soil to be moistened;
w = the weight of water to be applied.

Then $P \times W = w$ (1)

Since the quantity of water applied to a soil is usually expressed in depth over the surface, it is desirable to so express the quantity here needed.

Therefore let A = the area of land to be irrigated;
D = the depth of soil which needs water;
Vw = the volume weight of the soil;
d = the depth of water to be added;

Then $W = VwAD$ and $w = Ad \times 1$

Consequently, by substituting for W and w in equation one, their values as above, we have

$P \times VwAD = Ad$ and $d = P \times Vw \times D$ (2)

By taking D as 12 inches and assuming several values of Vw, figure 1 has been prepared from equation 2. For a given value of Vw the number of inches of water necessary to add a given percentage of moisture to 1 foot of soil may be readily determined.

EXPERIMENTAL CONDITIONS

The investigations here reported were made under the following conditions: First, on a number of alfalfa fields in Sacramento Valley representative of the best practice there; second, on 0.25-acre plots of the irrigation tract at the University of California farm at Davis; and third, on the 0.4-acre plots of a temporary experimental tract about 4 miles northeast of Willows, also in Sacramento Valley.

The soil of the typical farms ranges from silt loam underlain with fine sandy loam to heavy clays. The upper 2 feet of the Yolo loam in the

irrigation tract at the University farm is very uniform in texture; the third- to eighth-foot sections consist of fine sandy loam which is pocketed at irregular intervals with coarse sand and clay loam, below which is a heavy clay loam extending from 9 to 20 feet or more below the surface. The Tehema clay of the Willows tract is impervious to water and is very hard when dry.

Strictly speaking, the quantities of water accounted for, as given in the following pages, except as noted in the sixth and seventh columns of Table II, are a little low, since they do not include the water used by the plant immediately after irrigation. Upon the basis of experiments previously conducted at Davis (*6*), a total of approximately 1.08 inches of water was evaporated and used by the alfalfa during the first four

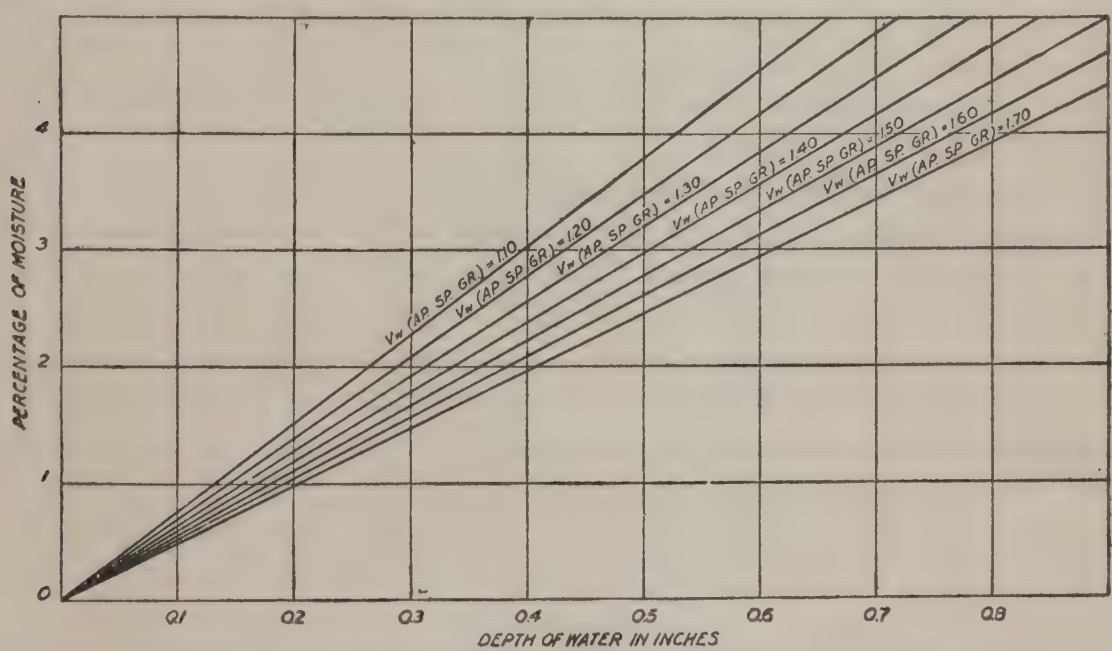

FIG. 1.—Diagram for determining the depth of irrigation water in inches necessary to add a given percentage of moisture to 1 foot of soil.

days after irrigation, the average time which elapsed between irrigation and the collecting of soil samples for moisture determinations. Including these probable evaporation losses, the total quantities and percentages of water accounted for are given in the sixth and seventh columns of Table II. When the average depth of irrigation water applied was small, as in the clay soils, the percentage loss of water during the first four days after irrigation was relatively high; consequently the percentages accounted for as given in the seventh column of Table II are relatively high for heavy soils, varying from 41.5 in the silt-loam soils to 69.4 in the clay soils. However, since practical considerations involved in irrigation farming demand that the water supply of the plant be furnished at different periods, the quantities of water stored in the soil by each irrigation, as indicated in column 5 of Table II (not including evaporation) and in Tables III and IV, may well be considered in each case representative of the effective irrigation. Moreover, since the studies

were all made in connection with the growth of but one crop (alfalfa), and as the time of observation after irrigation was made as nearly as possible the same, it is believed that the results are comparable. The acre-inch equivalents of the percentages before and after irrigation for each acre-foot of soil were calculated by using equation 2, deduced on page 3. The volume weights (Vw) used represent the density of the soil in place, except in a few cases as noted. In order to facilitate comparison with quantities of water applied in irrigation, and also with the total pore space of the soil, the quantities of moisture found before and after irrigation are presented in terms of acre-inches per acre-foot of soil.

PRECISION OF RESULTS

The precision of the results presented in this paper is dependent upon the following independent factors: (1) The accuracy of the moisture determinations, (2) the accuracy of the volume weight determinations, (3) the accuracy of the water measurements, and (4) the uniformity of lateral distribution of the irrigation water.

Factors 1 and 2 determine the precision of the absolute quantities of water retained by the soil, but the accuracy of the percentages of water accounted for depends upon all of the above factors. By use of Peter's formula[1] probable-error (*p. e.*) determinations for each foot of soil in 57 sets of six observations indicate average probable-error values for single observations (r) of ± 1.32 and ± 1.52 per cent of moisture before and after irrigation, respectively. The probable error of the mean of n determinations (ro) calculated from the relation:

$$ro = \frac{r}{\sqrt{n}}$$

indicates for the average number of borings per field or plot in which $n = 36$ average probable-error values of ± 0.22, and ± 0.25; for a type of soil n averages 114 and the probable-error values are $= \pm 0.12$ and ± 0.14, respectively, from which the probable error of the difference in moisture content before and after irrigation $= \pm 0.18$ per cent of moisture.

By using 1.40 as a mean volume weight, ± 0.18 per cent of moisture is equivalent to ± 0.03 acre-inch of water per acre-foot of soil. The average probable error of the volume-weight determinations (± 0.01) equivalent to $+0.002$ acre-inch of water per acre-foot of soil is too small to be significant. Hence, the average probable error of the increase in water due to irrigation $= \pm 0.03$ acre-inch for each acre-foot of soil. Upon this basis the chances are only 1 to 1 that increases of 0.03 inch for each foot of soil as given in the averages for the various types are due to the

[1] $r = 0.8453 \times \frac{\Sigma V}{\sqrt{n(n-1)}}$ where r = the probable error of a single observation;

$ro = 0.8453 \times \frac{\Sigma V}{n\sqrt{(n-1)}}$ ro = the probable error of the arithmetic mean of n observations;

ΣV = the sum of the residuals without regard to sign;

n = the number of observations.

application of water. Also the chances are 823 out of 1,000 that increases of 0.06 inch per foot are due to the irrigation, and 957 out of 1,000 that increases amounting to 0.09 inch were caused by irrigation.[1]

The probable error of the percentage of water accounted for can be only estimated since the water measurement data at hand do not make possible an accurate determination of the probable error of this factor. Moreover, it is dependent on uniform lateral surface distribution, which is, in fact, seldom attained. The field observations upon some of the tracts warrant the conclusion that lack of uniformity in lateral distribution [2] is responsible for the apparent discrepancies in the percentage of water accounted for.

A further test of precision was attempted by making three borings at one time, each within a distance of 6 feet of the other two, and calculating the mean deviation of each determination from the average of the three. Thirteen sets of such determinations to a depth of 6 feet gave an average deviation of ±0.62 per cent of moisture, an average probable error for a single observation of ±0.66 per cent of moisture, which is approximately one-half of the probable error found in connection with six determinations scattered over an entire field representing an area of approximately 5 acres.

RESULTS ON TYPICAL ALFALFA FIELDS

EXPERIMENTAL CONDITIONS

The soils of these fields are divided into four general classes, notwithstanding some variation in physical properties of different types of soil within one class. While examining the data presented in Tables III and IV, reference should be made to Table I, which contains the volume weights and moisture equivalents (*3*) of the soils considered. It is to be noted that the volume weights vary from 1.10 to 1.75 and that the silt-loam soils are lighter than the clay loams and clays. These observations are not in accord with the ideas generally entertained concerning the relation of volume weight to soil texture.

[1] It must be remembered that, because of variations in soil, number of borings, and other less important factors, these values of the probable error do not apply equally to each type of soil given. Where the number of borings differs greatly from 114 (the average used) a more accurate value of the probable error may be obtained by multiplying 0.03 by the ratio of the square roots of the mean, 114, and the number of borings made in a given type of soil. For example, the averages for the Willows experimental tract given at the bottom of Table IV are based upon 284 borings. The probable error of the increase is therefore ±0.019 inch per foot of soil. The minimum difference observed is 0.04 inch. The ratio of this difference to the average probable error of the difference is 2.1, which means that the chances are 843 out of 1,000 that the minimum difference was due to the irrigation.

[2] It is recognized that the degree of uniformity in lateral distribution which can be attained frequently determines the quantity of water applied per irrigation.

TABLE 1.—*Volume weight of the upper 6 feet of different soils at depths varying from 1 to 9 feet, together with the moisture equivalent. Observations made upon soils from typical alfalfa farms in Sacramento Valley, Cal., 1913, 1914, and 1915*

Name and location of tract	Volume weight of soil	How moisture equivalent is expressed	Moisture equivalent (%).									Average
Depth at which sample was taken ... feet			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
SILT LOAM SOILS HAVING FINE SANDY LOAM SUBSOILS												
Wilson, Los Molinos	[illegible]	Per cent	24.18	24.45	23.62	24.25	21.27	16.44				[illegible]
		Inches per foot of soil	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]				[illegible]
Griffin, Woodland	[illegible]	Per cent	22.64	21.22	20.78	19.89	16.7	18.98	22.18	22.11	24.46	[illegible]
		Inches per foot of soil	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	3.49	3.43	3.52	3.49
Average	[illegible]	do	[illegible]	3.14	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
SILT LOAM SOILS												
Bundy, Los Molinos	[illegible]	Per cent	28.63	26.90	27.00	27.03	27.96	29.96				[illegible]
		Inches per foot of soil	4.47	4.20	4.22	4.22	4.37	4.37				4.34
Beck, Woodland	[illegible]	Per cent	25.68	25.18	28.03	26.09	21.43					[illegible]
		Inches per foot of soil	4.07	3.99	4.44	4.21	3.40					4.02
Hollenbeck, Los Molinos	[illegible]	Per cent		22.14	19.87	16.54	20.99		20.13	24.72	22.69	[illegible]
		Inches per foot of soil		3.45	3.04	3.63	3.17		3.14	3.86	3.54	3.39
Average	[illegible]	do	4.28	3.88	3.90	3.69	3.68	4.37	3.24	3.86	3.54	3.88
SILT LOAM SOILS NOT INCLUDED IN ABOVE GROUP												
Hudson, Woodland	[illegible]	Per cent	22.16	21.79	21.43	21.62	26.23	27.34		28.66	24.86	24.18
		Inches per foot of soil	[illegible]	3.77	3.77	3.75	3.84	4.00		4.07	3.61	[illegible]
Huerkamp, Corning	[illegible]	Per cent	21.46	22.16	22.40	22.46	24.46	25.49				23.26
		Inches per foot of soil	3.48	3.48	3.53	3.98	3.58	4.78				4.02

CLAY-LOAM SOILS

O'Hair, Orland	[b] 1.33	Per cent	23.87	21.70	21.80	21.41	19.91	18.79				21.25
		Inches per foot of soil	3.18	2.89	2.90	2.85	2.65	2.50				2.83
Geer, Los Molinos	1.27	Per cent	24.75	26.71	25.90	26.54	27.76	27.64				26.55
		Inches per foot of soil	3.78	4.08	3.95	4.05	4.23	4.22				4.05
Guile, Woodland	1.40	Per cent	24.18	28.74	27.65	28.20	25.34	24.94				26.50
		Inches per foot of soil	4.07	4.83	4.65	4.73	4.26	4.20				4.43
Jackson-Woodard, Woodland	1.26	Per cent	30.31	31.65	31.79	29.92	28.70	29.14				30.25
		Inches per foot of soil	4.58	4.79	4.81	4.53	4.34	4.42				4.58
Wright, Dixon	[c] 1.50	Per cent	27.72	27.54	25.60	24.76	27.38					26.60
		Inches per foot of soil	4.98	4.96	4.61	4.46	4.93					4.79
Average	1.35	.....do	4.12	4.31	4.18	4.12	4.08	3.84				4.14

CLAY SOILS

Purdy, Willows	1.64	Per cent	21.02	28.62	34.20	25.65	32.44	32.52				29.08
		Inches per foot of soil	4.14	5.64	6.74	5.22	6.38	6.41				5.75
Tuttle, Willows	1.74	Per cent	19.30	20.38	20.79	23.68	24.85	27.48				22.75
		Inches per foot of soil	4.03	4.26	4.34	4.95	5.19	5.74				4.78
Average	1.69	.....do	4.08	4.95	5.54	5.08	5.78	6.08				5.25

CLAY SOILS SAMPLED AT DIFFERENT DEPTHS

Depth at which sample was taken.....inches			4	12	20	30	42	54				
Plots 3 and 4, Willows experimental tract	[d] 1.75 (1.778±0.006)	Per cent	19.70	20.38	22.64	21.98	22.09					21.36
		Inches per foot of soil	4.14	4.28	4.76	4.62	4.64					4.49
Plots 6 and 7, Willows experimental tract	[d] 1.75 (1.711±0.006)	Per cent	22.59	22.78	23.72	23.94	23.22					23.28
		Inches per foot of soil	4.74	4.78	4.97	5.03	4.88					4.88
Plots 11 and 12, Willows experimental tract	[d] 1.75 (1.743±0.017)	Per cent	20.70	21.10	22.74	23.76	25.60					22.78
		Inches per foot of soil	4.35	4.43	4.78	5.00	5.38					4.79
Average	1.75	.....do	4.41	4.50	4.84	4.88	4.97					4.72

[a] Based on 1913 field work as corrected by 1914 work on Bundy tract.
[b] Based on laboratory determinations with Bowman soil compactor.
[c] Work of 1913 corrected by 1913 and 1914 work on Jackson-Woodard tract.
[d] Average of determinations for entire field, 12 in number, used in calculations. Figures in parentheses are averages of three tests in the respective controls.

TABLE 12.—Number of irrigations per season and the average depths of water applied in each irrigation and retained by the upper 6 feet of soil for 17 typical alfalfa farms in Sacramento Valley, California, 1923, 1924, and retained by the upper 5 feet of soil on Willows experimental plot, 1925

Class of soil and name of farm	Location	Number of irrigations per season	Average depths of water applied per irrigation	Quantity of water retained by upper 6 feet of soil	Quantity of water retained, as that of probable evaporation loss	Percentage of average amount applied, retained by 6 feet of soil	Percentage of water retained, including probable evaporation loss
Silt-loam soils having fine sand-by-loam subsoils:			Inches	Inches	Inches		
Migna	Los Molinos	4	18.17	4.84	5.47	26.8	31.7
Crilles	Woodland	2	11.78	5.14	7.00	33.1	60.4
Average			15.00	5.50	6.60	[illegible]	43.2
Silt-loam soils:							
Bundy	Los Molinos	4	11.30	4.51	5.11	39.3	41.8
Dock	Woodland	1	9.52	4.19	5.07	44.0	53.3
Balbonke	Los Molinos	4	16.60	4.57	5.59	27.1	33.8
Average			10.81	4.24	5.38	[illegible]	41.5
Silt-loam soils not included in above group because of special conditions:							
Hughson	Woodland	4	29.50	8.00	4.18	22.7	41.8
Shannon	Chilley	[illegible]	[illegible]	4.11	5.47	58.1	73.1
Williams [illegible]	do	[illegible]	[illegible]	1.70	3.84	48.4	61.3
Clay-loam soils:							
O'Hair	Orland	1	4.21	1.65	3.71	65.7	89.7
Gent	Los Molinos	4	10.97	4.50	5.38	22.3	28.4
Gulla	Woodland	2	8.81	4.70	5.78	71.1	87.3
Jackson-Woodland	do	1	8.24	3.33	4.41	41.4	54.8
Bright	Dixon	[illegible]	5.44	1.17	3.39	41.4	60.3
Average			8.78	3.50	4.58	[illegible]	52.0
Clay soils:							
Purdy	Willows	4	5.06	2.58	4.70	23.1	32.8
Tuttle	do	[illegible]	4.18	2.31	4.21	80.4	47.4
Average			4.72	1.10	3.18	[illegible]	69.4
Clay soils, Willows experimental tract:							
Plots 1 and 4		17	4.00	2.54	1.86	47.0	80.0
Plots 5 and 7		8	1.00	1.27	1.13	35.7	71.2
Plots 1 and 10		4	8.00	1.57	1.15	46.9	44.3
Average			3.87	1.80	1.14	[illegible]	58.3

[illegible]

In Table II are presented the number of irrigations which each tract was given annually, and the average depths of water applied per irrigation. These were found to decrease rapidly with the increase in fineness of the soil texture, averaging 15.02 inches for the silt-loam soils having fine sandy-loam subsoils and only 4.72 inches for the clay soil. The quantity of water retained by the upper 6 feet of soil also decreased with increase in fineness of texture.

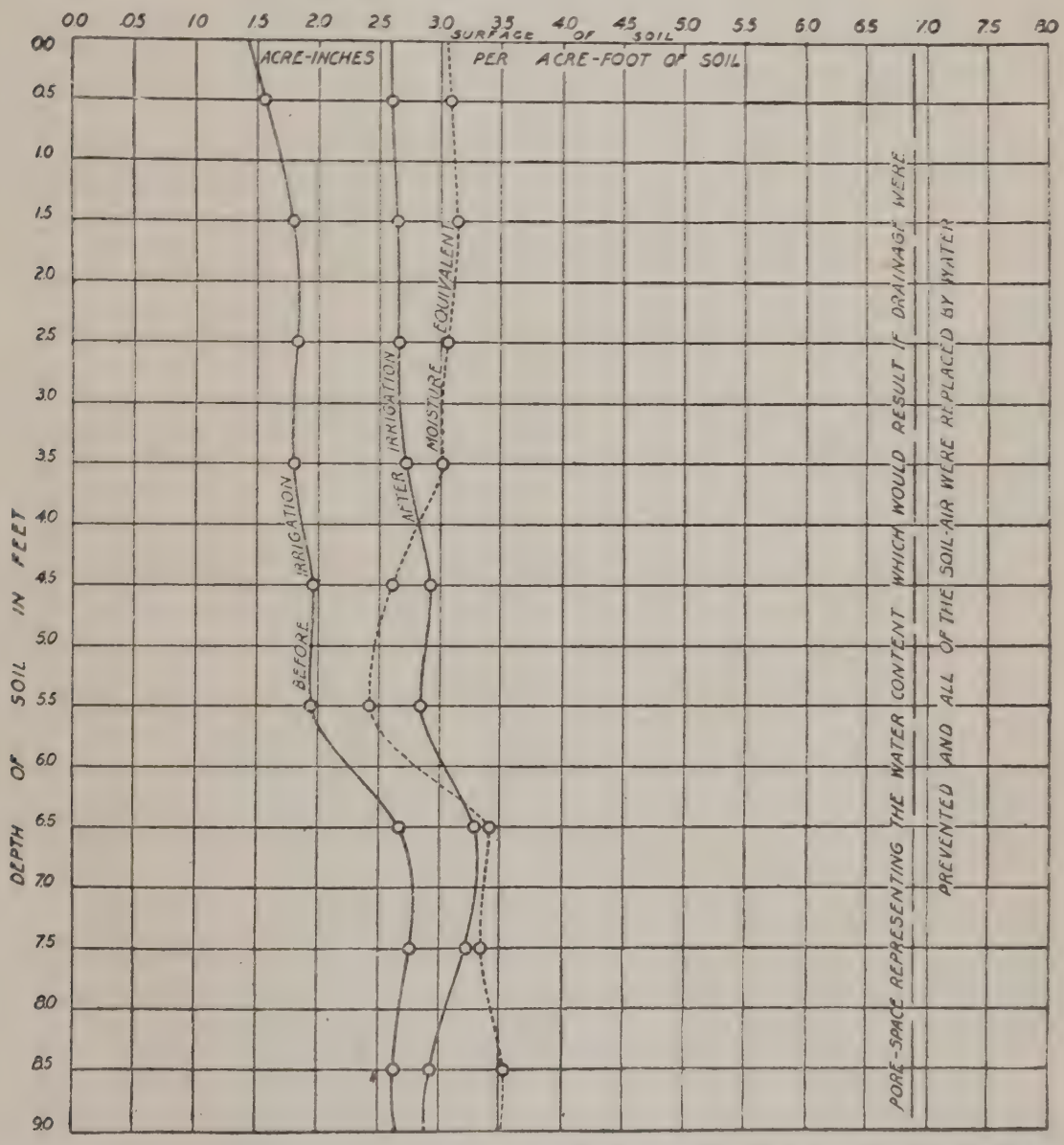

FIG. 2.—Graphs of the water content before and after irrigation, moisture equivalent, and pore space of silt-loam soils having fine sandy-loam subsoils. Each water-content curve is the average of 62 borings.

SILT-LOAM SOILS HAVING FINE SANDY-LOAM SUBSOILS

In Table III and figure 2 are presented the results of 108 6-foot and 16 9-foot borings, thus making a total of 792 moisture determinations. It is of special interest to note that when these soils contained all of the water they would hold against gravity, an average of 2.73 inches for for each of the upper 6-foot sections, only 40 per cent of the pore space of the soil was filled. Apparently the maximum water capacity is

fixed by the total external surface area of the soil particles, the individual pore spaces being so large as to prevent the water films about the particles from consolidating sufficiently to fill appreciably the interstices.

TABLE III.—*Water content before and after irrigation, the moisture equivalent, and the pore space of the soils of typical alfalfa farms in Sacramento Valley, California, 1914. Averages for each foot of soil to a depth of 9 feet* [a]

[Results expressed in acre-inches per acre-foot of soil]

SILT-LOAM SOILS HAVING FINE SANDY-LOAM SUBSOILS [b]

Tract, location, and time of sampling.	Number of borings for soil samples.	Water content.									Moisture equivalent.	Pore space.
Depth of soil at which samples were taken, feet	.....	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5		
Wigneau tract, Los Molinos:												
Before irrigation	26	1.77	2.00	1.97	2.02	2.28	2.04	[c]2.67	2.76	2.62	[d]2.95	7.10
After irrigation	26	2.62	2.73	2.77	2.85	3.08	2.93	[c]3.28	3.21	2.91		
Increase	.....	.85	.73	.80	.83	.80	.89	.61	.45	.29		
Griffes tract, Woodland:												
Before irrigation	36	1.34	1.60	1.69	1.59	1.64	1.84	.....	.....	.....	[d]2.83	6.66
After irrigation	36	2.58	2.57	2.56	2.60	2.77	2.76	.....	.....	.....		
Increase	.....	1.24	.97	.87	1.01	1.13	.92	.....	.....	.....		
Averages:												
Before irrigation	62	1.56	1.80	1.83	1.80	1.96	1.94	2.67	2.76	2.62	2.89	6.88
After irrigation	66	2.60	2.65	2.66	2.72	2.92	2.84	3.28	3.21	2.91		
Increase	.....	1.04	.85	.83	.92	.96	.90	.61	.45	.29		

SILT-LOAM SOILS [e]

Bundy tract, Los Molinos:												
Before irrigation	24	2.54	2.86	2.84	2.84	2.97	3.12	3.19	3.50	3.50	[d]4.34	6.31
After irrigation	24	3.50	3.62	3.61	3.52	3.42	3.53	3.57	3.82	3.90		
Increase	.....	.96	.76	.77	.68	.45	.41	.38	.32	.40		
Beck tract, Woodland:												
Before irrigation	18	1.72	2.09	2.12	2.19	2.48	2.83	.....	.....	.....	4.02	6.18
After irrigation	18	2.97	2.84	2.83	2.85	3.01	3.12	.....	.....	.....		
Increase	.....	1.25	.75	.71	.66	.53	.29	.....	.....	.....		
Hofhenke tract, Los Molinos:												
Before irrigation	45	2.12	2.43	2.37	2.37	2.44	2.49	2.72	2.99	3.04	[f]3.29	6.32
After irrigation	45	3.27	3.29	3.04	3.00	3.04	3.09	3.26	3.30	3.35		
Increase	.....	1.15	.86	.67	.63	.60	.60	.54	.31	.31		
Averages:												
Before irrigation	87	2.13	2.46	2.44	2.47	2.63	2.81	2.96	3.24	3.27	3.88	6.27
After irrigation	87	3.25	3.25	3.16	3.12	3.16	3.25	3.42	3.56	3.62		
Increase	.....	1.12	.79	.72	.65	.53	.44	.46	.32	.35		

[a] See Table II for the number of times each of the above fields was irrigated, the average quantities of water applied, and the total amounts of water retained by the soil.
[b] These data are presented graphically in figure 2.
[c] From 7 to 9 feet average of only 8 borings.
[d] Upper 6 feet.
[e] These data are presented graphically in figure 3.
[f] Upper 9 feet, less first and sixth.

SILT-LOAM SOILS

In Table III and figure 3 are presented results of moisture determinations upon three tracts classed as silt-loam soils which are based upon 138 6-foot and 36 9-foot borings, making a total of 1,152 moisture determinations. The results for the upper 6 feet of soil as presented in figure 3

are based upon 174 borings and 1,044 moisture determinations, while those of the depth from 7 to 9 feet are based upon 36 borings and 108 single observations.

The curves of the silt-loam soils converge from the surface of the soil downward. This seems to be due in large measure to the fact that these soils do not dry out as rapidly at great depths as do the more porous fine

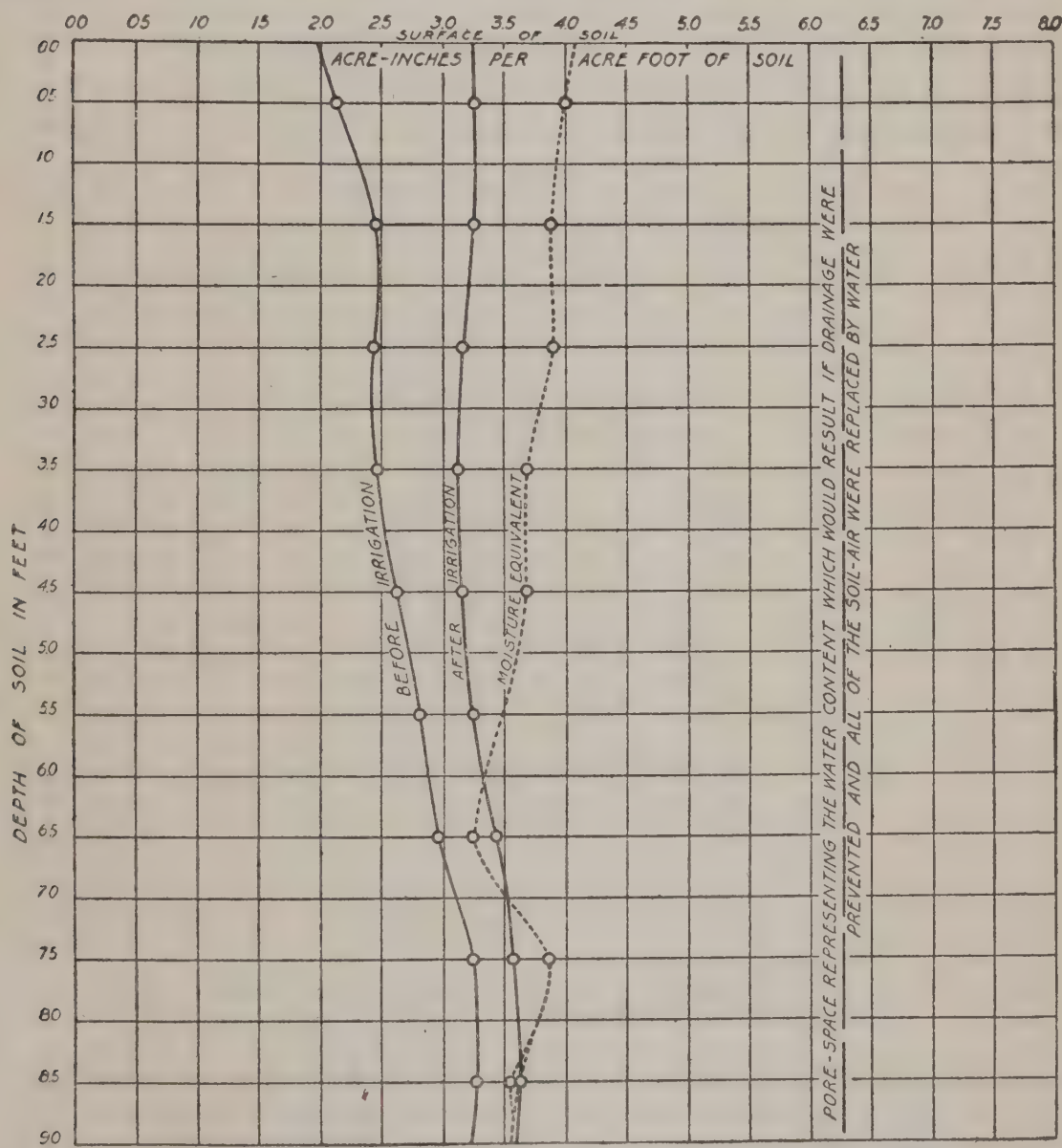

FIG. 3.—Graphs of the water content before and after irrigation, moisture equivalent, and pore space of silt-loam soils. Each water-content curve is the average of 87 borings.

sandy-loam subsoils, since the observations after irrigation show very little decrease of water content with depth. As the soils are very uniform, it seems reasonable to conclude that their maximum capillary capacities were satisfied. The average amount of water held after irrigation was 3.20 inches per foot, or enough to fill 51 per cent of the pore space, as compared to 2.73 inches per foot, or enough to fill 40 per cent of the pore space for the loam soils having fine sandy loam subsoils.

CLAY-LOAM SOILS

In Table IV and figure 4 are given the quantities of water held before and after irrigation for five typical farms having clay loam soils as determined by 296 six-foot borings, making 1,776 single observations.

Figure 4 represents average results of the five clay-loam fields. The increase in water content varies from 1.35 in the surface to 0.28 in the sixth foot, as compared to a variation of 1.13 to 0.44 in the silt-loam soils and 1.04 to 0.90 in the silt loams having fine sandy-loam subsoils. The increase in convergence of curves with depth as the texture of the soil increases in fineness is to be noted. The water content after irri-

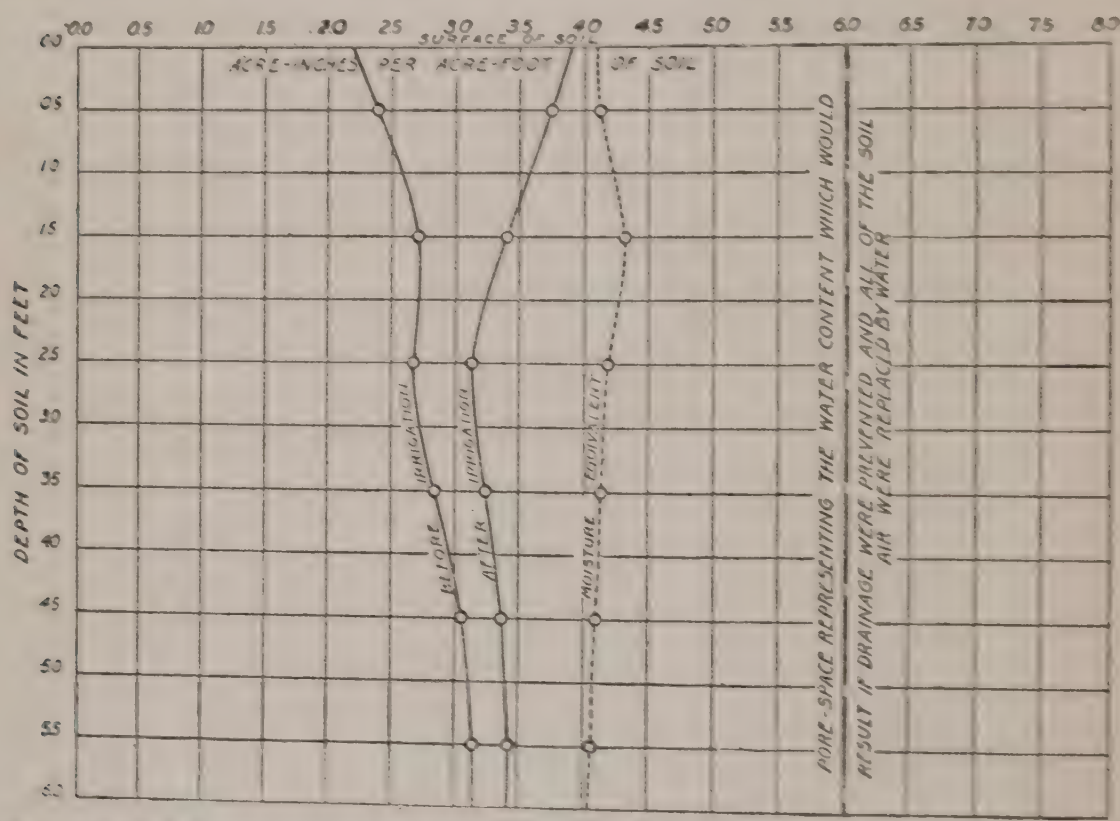

FIG. 4.—Graphs of the water content before and after irrigation, moisture equivalent, and pore space of clay-loam soils. Each water-content curve is the average of 148 borings.

gation seems to decrease appreciably with depth of soil. It is therefore doubtful if the maximum capillary capacities of these soils were satisfied. The average amount of water held by the clay loams after irrigation was 3.49 inches per foot, or enough to fill 58 per cent of the pore space as compared to 40 and 51 per cent, respectively, in the first and second types of soil considered.

CLAY SOILS

It was pointed out above that the maximum water-holding powers of clay soils are sometimes limited by their pore space. This condition seems to apply to the soils described below, the volume weights of which were found to be unusually high. The total external surface area of

these soils is in all probability very high, to judge from their mechanical analysis, which showed 24.54 per cent of total sands, 40 per cent of silt, and 34.84 per cent of clay. Yet the quantities of water found in them both before and after irrigation were extremely low. This condition is especially evident when the results are recorded in percentages rather than in inches per foot, since the high volume weights increase their water content relatively when reported upon the latter basis.

The observations made upon clay soils are presented in Table IV and figures 5 and 6. Figure 5 is based upon 86 six-foot borings, making 516 moisture determinations, and figure 6 contains averages of 568 borings

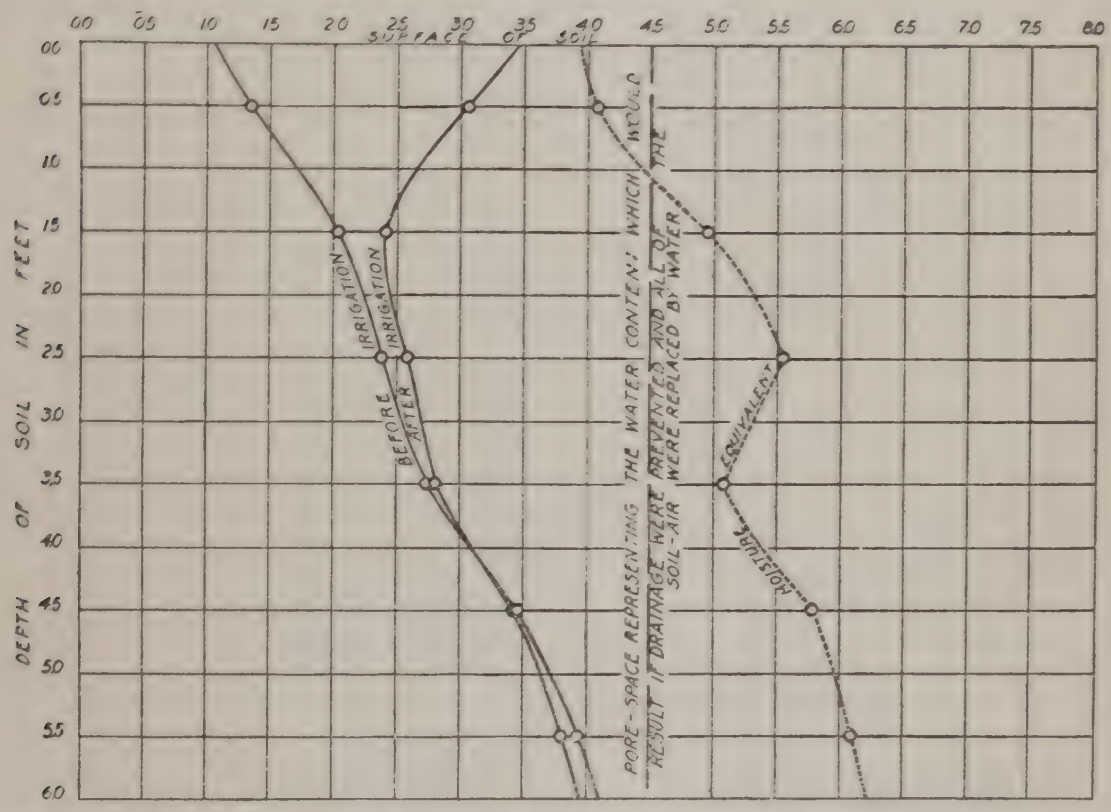

FIG. 5.—Graphs of the water content before and after irrigation, moisture equivalent, and pore space of clay soils. Each water-content curve is the average of 43 borings.

in which 3,408 moisture determinations were made. Table IV reveals at a glance the striking fact that only the surface foot of soil was appreciably moistened by the irrigation water. It is doubtful if the capillary power of the surface foot was entirely satisfied; yet it held after irrigation 3.06 inches of water, or enough to fill 64.3 per cent of its pore space. The sixth foot, which was kept moist by the ground-water table, contained no gravitational water, but 86 per cent of its pore space was occupied by capillary water, leaving only 16 per cent of the 4.71 inches of pore space per foot of soil for air, or only two-thirds of one inch in twelve.

TABLE IV.—*Water content before and after irrigation, the moisture equivalent and the pore space of the soils of typical alfalfa farms in Sacramento Valley, California, 1913–1915. Averages for each foot of soil to a depth of 6 feet* [a]

[Results expressed in acre-inches per acre-foot of soil]

CLAY-LOAM SOILS, 1913–14 [b]

Tract, location, and time of sampling.	Number of borings for soil samples.	Water content.						Moisture equivalent in acre-inches per acre-foot of soil.	Pore-space acre-inches per acre-foot of soil.
Depth of soil at which samples were taken	.....	0.5	1.5	2.5	3.5	4.5	5.5		
O'Hair, Orland:									
Before irrigation	27	1.46	1.83	2.07	2.69	3.21	3.51	3.39	6.10
After irrigation	27	3.14	2.51	2.40	2.71	3.21	3.44		
Increase	.....	1.68	.68	.33	.03	.00	—.07		
Geer, Los Molinos:									
Before irrigation	48	2.60	3.06	2.40	2.44	2.66	2.67	4.05	6.36
After irrigation	48	3.67	3.86	3.07	3.17	3.27	3.21		
Increase	.....	1.07	.80	.67	.73	.61	.54		
Guile, Woodland:									
Before irrigation	12	2.23	2.46	2.60	2.67	2.74	2.72	4.44	5.78
After irrigation	12	4.00	3.34	3.18	3.22	3.20	3.18		
Increase	.....	1.77	.88	.58	.55	.46	.46		
Jackson-Woodard, Woodland:									
Before irrigation	36	2.46	2.79	2.92	3.02	3.12	3.25	4.57	6.42
After irrigation	36	4.04	3.52	3.28	3.28	3.31	3.46		
Increase	.....	1.58	.73	.36	.26	.19	.21		
Wright, Dixon:									
Before irrigation	25	3.21	3.40	3.31	3.29	3.41	3.47	4.79	5.38
After irrigation	25	3.83	3.76	3.69	3.70	3.72	3.70		
Increase	.....	.62	.36	.38	.41	.31	.23		
Averages:									
Before irrigation	148	2.39	2.71	2.66	2.82	3.03	3.12	4.25	6.01
After irrigation	148	3.74	3.40	3.12	3.22	3.34	3.40		
Increase	.....	1.35	.69	.46	.40	.31	.28		

CLAY SOILS, 1914 [c]

Purdy, Willows:									
Before irrigation	24	1.30	1.99	2.39	2.82	3.71	4.36	5.73	4.71
After irrigation	24	3.03	2.12	2.51	2.79	3.54	4.06		
Increase	.....	1.73	.13	.12	—.03	—.17	—.30		
Tuttle, Willows:									
Before irrigation	19	1.40	2.09	2.36	2.64	3.16	3.48	4.75	4.25
After irrigation	19	3.09	2.70	2.65	2.81	3.29	3.52		
Increase	.....	1.69	.61	.29	.17	.13	.04		
Averages:									
Before irrigation	43	1.35	2.04	2.38	2.73	3.44	3.92	5.24	4.48
After irrigation	43	3.06	2.41	2.58	2.80	3.42	3.79		
Increase	.....	1.71	.37	.20	.07	—.02	—.13		

[a] See Table II for the number of times each of the above fields were irrigated, the average quantities of water applied, and the total amounts of water retained by the soil.

[b] These data are presented graphically in figure 4.

[c] The plan of sampling the upper 2 feet of the clay soils was in 1915 changed from one sample in the middle of each foot-section to one in the middle of each 8-inch section, thus giving three samples in the upper 2 feet. The sixth foot was not sampled in 1915. These data are presented graphically in figures 5 and 6.

TABLE IV.—*Water content before and after irrigation, the moisture equivalent and the pore space of the soils of typical alfalfa farms in Sacramento Valley, California, 1913–1915. Averages for each foot of soil to a depth of 6 feet*—Continued

CLAY SOILS, 1915 [a]

Tract, location, and time of sampling.	Number of borings for soil samples.	Water content.						Moisture equivalent in acre-inches per acre-foot of soil.	Pore-space acre-inches per acre-foot of soil. memm
Depth at which samples were taken, inches.	.....	4	12	20	30	42	54		
Plots 3 and 4, Willows experimental tract:									
Before irrigation	140	1.71	2.10	2.41	2.61	2.87	3.96	4.49	4.18
After irrigation	140	2.40	2.18	2.42	2.63	2.84	3.99		
Increase	.....	.69	.08	.01	.02	—.03	.03		
Plots 6 and 7, Willows experimental tract:									
Before irrigation	96	1.78	2.31	2.67	2.99	3.36	4.95	4.88	4.49
After irrigation	96	2.97	2.62	2.79	3.06	3.38	4.85		
Increase	.....	1.19	.31	.12	.07	.02	—.10		
Plots 11 and 12, Willows experimental tract:									
Before irrigation	48	1.45	1.97	2.38	2.62	2.93	4.08	4.78	4.35
After irrigation	48	2.91	2.52	2.50	2.73	3.06	3.99		
Increase	.....	1.46	.55	.12	.11	.13	—.09		
Averages:									
Before irrigation	284	1.65	2.13	2.49	2.74	3.05	4.33	4.72	4.34
After irrigation	284	2.76	2.44	2.57	2.81	3.09	4.28		
Increase	.....	1.11	.31	.08	.07	.04	—.05		

[a] The plan of sampling the upper 2 feet of the clay soils was in 1915 changed from one sample in the middle of each foot-section to one in the middle of each 8-inch section, thns giving three samples in the upper 2 feet. The sixth foot was not sampled in 1915. These data are presented graphically in figures 5 and 6.

The unusual conditions which were encountered in the Willows area during 1914 seemed to warrant further work upon this type of soil, and in 1915 twelve 2-inch, eight 4-inch, and four 6-inch irrigations were given to plots 3 and 4, 6 and 7, and 11 and 12, respectively, of the Willows experimental tract.

Three samples were taken in the upper 2 feet of soil—that is, one representing each 8-inch section.[1] The relatively small individual increases observed in plots 3 and 4, as compared to those in plots 6 and 7, are probably due in part to differences in soil compactness,[2] but the smaller unit application of water was in all probability the chief controlling factor. The total seasonal increase in the different plots—that is, the unit increase multiplied by the number of irrigations as shown in Table V—is relatively higher in plots 3 and 4.

[1] In examining the water contents presented in Table IV, it must be remembered that the results presented in the three columns at the reader's left are at the rate of the various numbers of acre-inches per acre-foot of soil, and must therefore be multiplied by 8/12 to get the actual increase in each of the individual 8-inch sections. This has been taken into account in the calculations of quantities of water accounted for, as presented at the bottom of Table II.

[2] The soil of plots 6 and 7 is less compact than that of the other plots. (See Table I.)

TABLE V.—*Total seasonal increases in irrigation-water content of the Willows experimental tract*

Plot No.	Number of irrigations.	Unit increase in water content.	Total seasonal increase.
		Inches.	*Inches.*
3 and 4	12	0.54	6.48
6 and 7	8	1.07	8.56
11 and 12	4	1.57	6.28

The averages presented at the bottom of Table IV and in figure 6 show, like those of the Purdy and Tuttle tracts, that but very little water passed below the surface foot of soil.

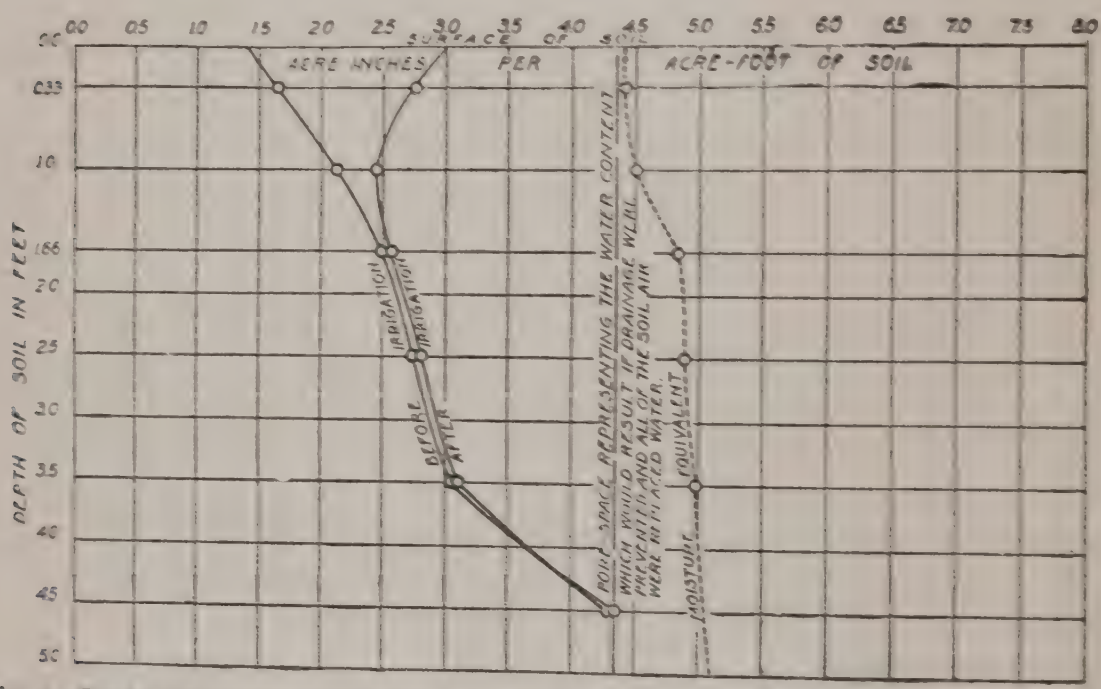

FIG. 6.—Graphs of the water content before and after irrigation, moisture equivalent, and pore space of clay soils. Each water-content curve is the average of 164 borings.

RESULTS ON UNIVERSITY FARM, DAVIS

Among other reasons, the systematic plan of irrigation followed and the length of time covered in the Davis work make it desirable to present separately the results there obtained. No appreciable differences in volume weight were found, notwithstanding textural differences, the average volume weight of several observations being 1.28. Upon this value the calculations given in the following tables are based. The moisture-equivalent determinations as presented in Table VI were made of samples from each of the upper 12 feet of soil taken from single borings in three plots,[1] D, F, and H. Plots B, C, D, E, F, and G[2] are sufficiently alike in texture to be placed in one group, although some varia-

[1] See Table VII for explanatory note on the numbering of the plots.
[2] As plot A was given no irrigation water, it is not reported in this paper.

tion exists, especially in the depths from 4 to 9 feet. Averages of the moisture equivalents of plots D and F are used for this group, including plots B to G, but the individual determinations are given in Table VI to show the variation of texture which exists and for which due allowance must be made.

TABLE VI.—*Moisture equivalents for soils at the University Farm, Davis, Cal., at depths varying from 1 to 12 feet*

[Results expressed in terms of percentage by weight and of acre-inches per acre-foot of soil]

Depth of soil in feet.	Plot D (per cent).	Plot F (per cent).	Average of plots D and F.[a]		Plot H.	
			Per cent.	Acre-inches per acre-foot of soil.	Per cent.	Acre-inches per acre-foot of soil.
0.5	23.32	24.44	23.88	3.67	28.41	4.37
1.5	22.00	21.80	21.90	3.37	31.70	4.87
2.5	21.24	15.96	18.60	2.86	34.02	5.23
3.5	18.93	11.69	15.31	2.36	30.47	4.69
4.5	16.60	21.52	19.06	2.93	31.38	4.82
5.5	14.08	16.90	15.51	2.39	27.23	4.19
6.5	14.03	23.84	18.94	2.91	29.36	4.51
7.5	15.28	26.90	21.09	3.24	26.92	4.13
8.5	12.07	23.12	17.60	2.71	26.90	3.98
9.5	20.44	21.36	20.90	3.22	23.22	3.57
10.5	26.32	24.50	25.41	3.91	20.80	3.20
11.5	26.36	24.36	25.36	3.90	24.77	3.81

[a] The average value of the moisture equivalent as determined upon samples of soil from plots D and F are used to represent the moisture equivalent in plots B, C, D, E, F, and G.

The soil of plot H, classed by the Bureau of Soils of the United States Department of Agriculture as a Yolo loam, is distinctly finer in texture than that of the other plots, excepting a part of plot B. The differences in texture between it and the other plots are most readily appreciated by examination of the moisture equivalents of the two soils.[1] Averages of the results of three years' observations, representing the number of inches and also the percentages of the quantities of water applied which were retained by the upper 6 feet and the upper 12 feet of soil, are given in Table VII.[2] Assuming, upon the basis of the evaporation and transpiration experiments cited (*6*), that 0.7 inch of water was lost by evaporation and transpiration in the two days which elapsed between the time of irrigation and of sampling, plots B, C, and D retained in the upper 6 feet of soil an average of about nine-tenths of the water applied. Plots E, F, and G retained in the upper 6 feet nearly seven-tenths of the water applied, and the entire amount was apparently retained by the upper 12 feet. Likewise, plot H retained two-thirds of the water applied in the upper 6 feet of soil and nine-tenths in the upper 12 feet.

[1] Plot B, which lies nearest plot H, forms the division line between the two types of soil above mentioned.
[2] See (*1, p. 10*) for a discussion of Table VII.

TABLE VII.—*Number of acre-inches and percentage of the amount of water applied in each irrigation which was retained by the upper 6 feet and the upper 12 feet of soil, University of California farm, Davis, Cal. Averages for 1913, 1914, 1915*

Plot No.	Number of irrigations per season.	Depth of water applied at each irrigation.	Amount retained in upper 6 feet of soil.		Amount retained in upper 12 feet of soil.	
			Inches.	Percentage of quantity applied.	Inches.	Percentage of quantity applied.
		Inches.				
B (18, 29) [a]	2	6.0	3.81	63.5	([b])	.....
C (19, 28)	3	6.0	5.52	92.0	.....	.....
D (20, 27)	4	6.0	4.43	73.8	.....	.....
Average of 6-foot tests, plots B, C, and D	3	6.0	4.59	76.4	.....	.....
E (21, 26)	4	7.5	5.10	68.0	8.00	106.6
F (22, 25)	4	9.0	5.49	61.0	9.56	106.1
G (23, 24)	4	12.0	6.68	55.6	10.32	86.0
Average of 12-foot tests, plots E, F, and G	4	9.5	5.76	[b] 60.6	9.29	[c] 97.7
H (31)	4	15.0	9.24	61.6	13.09	87.2

[a] For convenience in referring to the plots, letters are used instead of the numbers in parentheses. The latter were used on page 10, California Department of Engineering Bulletin 3.
[b] Borings were made only to a depth of 6 feet in plots B, C, and D.
[c] Group averages obtained by giving plots percentages and weights proportional to quantities of water applied.

The amounts of water held by each foot of soil before and after irrigation and the average increases which were found for the various treatments are set forth in Table VIII. For ease of comparison these data are presented graphically in figures 7 to 12. The water contents of plots B and D are compared in figure 7. The soil of plot B being finer in texture than that of plot D, especially below 3 feet, accounts for the higher water content both before and after irrigation. Variation in the soil of plot B is probably the cause of an apparent discrepancy in the relative amounts of water accounted for. The comparison of plots C and D presented in figure 8 indicates that plot C became drier before irrigation than did plot D, which accounts for the greater amount of water being retained by plot C since each plot contained about the same quantity after irrigation. Figure 9 indicates that the upper 6 feet of plots E and G were moistened to their full capillary capacity. It is likely that the clay-loam stratum of the seventh foot in plot G by retarding downward movement caused some gravitational water to be held in the fifth and sixth foot section till the time of sampling. The effect of the large irrigations of plot G is evident in the great difference between the moisture contained, before and after irrigation, in the third to the sixth foot sections. Figure 10 shows that the 12-inch irrigations of plot G caused slightly greater increases than the 9-inch applications of plot F in the second to seventh foot, below which the increases were very irregular.

TABLE VIII.—*Water content before and after irrigation of soils, University of California Farm, Davis, Cal. Average in each foot of soil to a depth of 12 feet for the years 1913, 1914, and 1915*[a]

[Results expressed in acre-inches per acre-foot of soil]

Plot number and irrigation treatment.	Time of sampling.	Number of borings.	Water content.												Total water at depths of—			See fig.
															0–6 ft.	7–12 ft.	0–12 ft.	
Depth at which samples were taken.....feet..			0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5				
B, 2–6-inch irrigation.....	Before irrigation.....	14	1.78	2.03	2.14	2.46	2.37	2.13	.....	.....	.....	.....	.....	.....	12.91	.....	.....	7
	After irrigation.....	14	3.26	2.99	2.86	2.69	2.73	2.19	.....	.....	.....	.....	.....	.....	16.72	.....	.....	
	Increase.....	.....	1.48	.96	.72	.23	.36	.06	.....	.....	.....	.....	.....	.....	3.81	.....	.....	.....
C, 3–6-inch irrigation.....	Before irrigation.....	21	1.68	1.93	1.91	1.80	1.43	1.29	.....	.....	.....	.....	.....	.....	10.04	.....	.....	8
	After irrigation.....	21	3.34	2.99	2.82	2.45	2.10	1.86	.....	.....	.....	.....	.....	.....	15.56	.....	.....	.....
	Increase.....	.....	1.66	1.06	.91	.65	.67	.57	.....	.....	.....	.....	.....	.....	5.52	.....	.....	.....
D, 4–6-inch irrigation.....	Before irrigation.....	28	1.76	2.06	2.06	1.87	1.59	1.49	.....	.....	.....	.....	.....	.....	10.83	.....	.....	7
	After irrigation.....	28	3.30	3.06	2.75	2.26	2.02	1.87	.....	.....	.....	.....	.....	.....	15.26	.....	.....	8
	Increase.....	.....	1.54	1.00	.69	.39	.43	.38	.....	.....	.....	.....	.....	.....	4.43	.....	.....	.....
E, 4–7.5-inch irrigation...	Before irrigation.....	28	1.95	2.16	2.08	1.85	1.73	1.63	1.47	2.81	3.24	3.63	4.25	5.10	11.40	20.53	31.93	9
	After irrigation.....	28	3.20	3.16	2.95	2.60	2.40	2.19	2.21	3.64	4.18	4.22	4.52	4.66	16.50	23.43	39.93	
	Increase.....	.....	1.25	1.00	.87	.75	.67	.56	.74	.83	.94	.59	.27	.44	5.10	2.90	8.00	.....
F, 4–9-inch irrigation.....	Before irrigation.....	28	1.85	2.04	1.92	1.76	1.87	1.59	2.56	3.38	2.84	4.04	4.53	4.69	11.03	22.04	33.07	10
	After irrigation.....	28	3.17	3.10	2.82	2.52	2.57	2.34	3.33	4.08	3.98	4.49	5.08	5.15	16.52	26.11	42.63	
	Increase.....	.....	1.32	1.06	.90	.76	.70	.75	.77	.70	1.14	.45	.55	.46	5.49	4.07	2.56	.....
G, 4–12-inch irrigation....	Before irrigation.....	28	1.91	2.18	2.05	1.96	2.11	2.32	3.37	3.16	2.94	3.88	3.86	4.46	12.53	21.67	34.20	9
	After irrigation.....	28	3.24	3.30	3.04	2.99	3.23	3.41	4.22	3.77	3.76	4.32	4.32	4.92	19.21	25.31	44.52	10
	Increase.....	.....	1.33	1.12	.99	1.03	1.12	1.09	.85	.61	.82	.44	.46	.46	6.68	3.64	10.32	.....
Average.....	Before irrigation...	147	1.82	2.07	2.02	1.95	1.85	1.74	2.47	3.12	3.01	3.85	4.21	4.75	11.46	21.40	32.86	11
	After irrigation....	147	3.25	3.10	2.87	2.58	2.51	2.31	3.26	3.83	3.97	4.34	4.64	4.91	16.63	24.95	41.58	
	Increase.....	.....	1.43	1.03	.85	.63	.66	.57	.79	.71	.97	.49	.43	.16	5.17	3.55	8.72	.....
H, 4–15-inch irrigation....	Before irrigation...	28	2.30	2.76	3.77	3.67	3.82	3.92	4.16	4.03	3.86	3.92	3.92	3.78	20.24	23.67	43.91	12
	After irrigation....	28	3.89	5.01	5.24	5.30	4.98	5.06	4.90	4.71	4.72	4.42	4.41	4.36	29.48	27.52	57.00	
	Increase.....	.....	1.59	2.25	1.47	1.63	1.16	1.14	.74	.68	.86	.50	.49	.58	9.24	3.85	13.09	.....

[a] In 1913 samples were taken only to a depth of 6 feet, and in 1915, 9 feet was the maximum depth of boring. Therefore results given for depths of 7 to 9 feet, inclusive, are based upon two years' work, and from 10 to 12 feet upon only one year's work.

The upper 6 feet of the water-content curves in figure 11 plotted from the averages in Table VIII are based on 294 borings and 1,764 moisture determinations; the section from 7 to 9 feet is based upon 120 borings and 360 single observations; and the depth from 9 to 12 feet represents averages of 48 borings and 144 moisture determinations. The curves for the upper 6 feet of plot H presented in figure 12 are based on 56 borings and 336 single observations; the section from 7 to 9 feet on 40 borings and 120 single determinations; and for the depth from 10 to 12 feet on 16 borings and 48 single moisture tests.

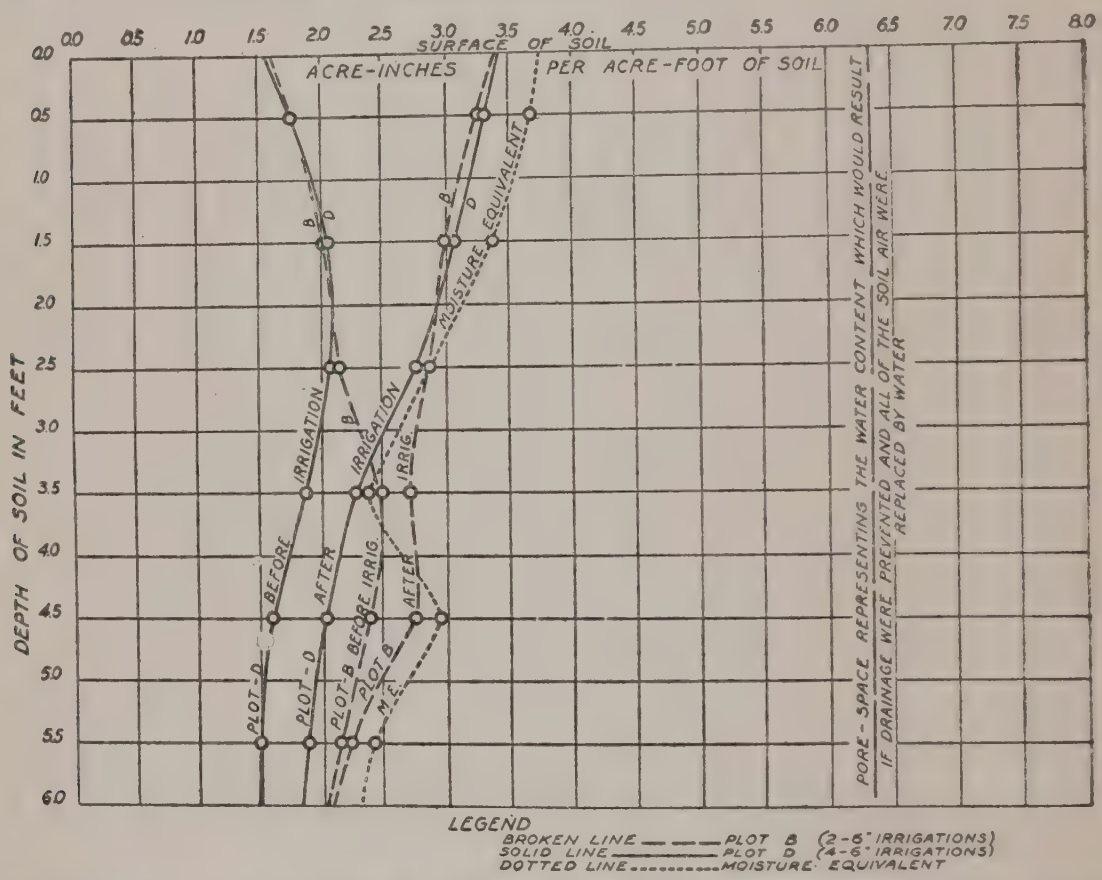

FIG. 7.—Graphs showing the comparison of water content before and after irrigation, moisture equivalent, and pore space of soils of plots B and D, Davis, Cal. Each water-content curve in plot B is the average of 14 borings; in plot D, of 28 borings.

MAXIMUM CAPILLARY CAPACITY OF DISTURBED SOILS AND SOILS IN PLACE

That the results of standard laboratory determinations of maximum capacity of soils do not accurately represent capillary capacities of soils in place is generally recognized. Widtsoe (*12*) determined the maximum capillary capacity of a soil in place by studying three columns 1 foot, 2 feet, and 3 feet long, respectively. The samples were taken by driving iron cylinders into the soil, thus getting it almost in its natural condition. By means of three independent linear equations, the effect of the special "lifting power" exerted by the end of each soil column was eliminated. It was found by this procedure that 28.4 per cent of the total water held in the 1-foot column was retained by this special force, which does not exist in ordinary field soils. The maximum capillary capacity thus

found was 26.8 per cent and the maximum quantity of water found in the soil after irrigation was 24.0 per cent.

It is highly desirable to establish, if possible, a relation between some of the more generally used soil constants (3) and the maximum field capacity of the soil. An attempt has therefore been made to compare the maximum capillary capacity as determined in the laboratory to the field capacity. The laboratory determinations were made by the use of brass cylinders 2 inches in diameter and 12 inches long, having perforated bottoms, which were filled on the Bowman soil compactor, placed in water to a depth of 10 inches overnight, and drained for 24 to 48 hours.

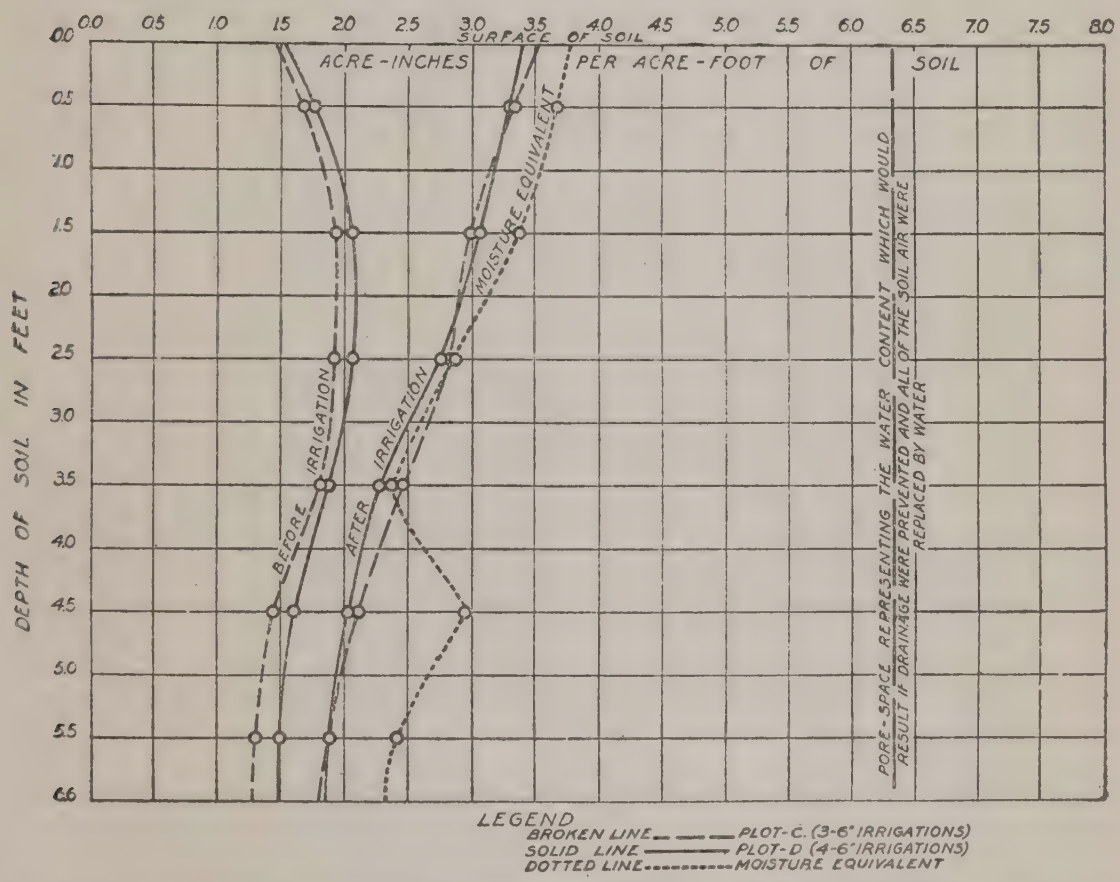

FIG. 8.—Graphs showing the comparison of water content before and after irrigation, moisture equivalent, and pore space of soils of plots C and D, Davis, Cal. Each water-content curve in plot C is the average of 21 borings; in plot D, of 28 borings.

The results reported in Table IX include the hygroscopic water contained in the air-dry sample used.

The surface foot of the first group of soils listed in Table IX was no doubt fully saturated by the large amounts of water applied. The quantities held per foot of soil vary from 2.58 in the Griffes tract to 3.67 in the Geer tract. The ratio of the amount held in the laboratory to that held in the field varies from 1.53 to 2.10 and has a mean of 1.78 ± 0.06. The probable error of a single observation is ± 0.19 inch per foot. Although the second group of soils was irrigated more moderately, the average unit application was 8.11 inches, which is much more than 1 foot of soil can retain. Therefore the amounts held

should closely approach the maximum capillary field capacity. The higher average ratio found, 1.98±0.14, is due in part to the fact that the soils are heavier and therefore undergo greater change in structure when disturbed. The probable error of the ratio for a single observation is ±0.24.

TABLE IX.—*Comparison of the maximum capillary capacities of disturbed soils as determined in the laboratory with the maximum quantities of water contained after irrigation by the surface foot of the soils in place*

Degree of irrigation.	Name and location of tract.	Maximum in laboratory (inches per foot of soil).	Maximum after irrigation (inches per foot of soil).	Ratio, laboratory to field capacity.	Pore space of soils in place (inches per foot of soil).	Ratio, laboratory capacity to pore space.
						Per cent.
Heavy	Wigno, Los Molinos	4.00	2.62	1.53	7.10	56.4
	Griffes, Woodland	4.90	2.58	1.90	6.66	73.5
	Bundy, Los Molinos	6.28	3.50	1.79	6.31	99.5
	Hofhenke, Los Molinos	5.02	3.27	1.54	6.32	79.5
	Hughson, Woodland	5.61	2.87	1.95	6.66	84.2
	Huartson, Gridley	5.93	2.92	2.03	6.06	98.0
	O'Hair, Orland	5.88	3.14	1.87	6.10	96.4
	Geer, Los Molinos	4.83	3.67	1.32	6.36	76.0
	University Farm, Davis	6.52	3.10	2.10	6.32	103.0
	Average	5.44	3.07	1.78 ±0.06	6.43	85.4
Medium	Guile, Woodland	7.64	4.00	1.91	5.78	132.0
	Jackson-Woodward, Woodland	6.97	4.04	1.72	6.42	108.0
	Beck, Woodland	6.85	2.97	2.30	6.18	111.0
	Average	7.15	3.67	1.98 ±0.14	6.13	117.0

In Table X comparisons are made between the maximum quantities contained after irrigation by the first- and second-foot sections of soil and their moisture equivalents (3). Examination of the ratio of the moisture equivalent to the maximum quantity of water held after irrigation indicates the former to be slightly larger than the latter. The averages of this ratio, 1.13±0.02 and 1.12±0.02, for the first- and second-foot sections, respectively, of the heavily irrigated soils show remarkable agreement.[1] The increase in the average ratio of the second group from 1.20±0.06 to 1.38±0.02 is due to the fact that the heavier soils of the second group were not so fully wetted in the second foot. Likewise, the average ratio 1.48±0.05 for the third group is high because of

[1] The ratio of the moisture equivalent to the moisture content, one week after irrigation, of various soils of Montana and Idaho has been determined by Prof. S. T. Harding, of the University of California, while connected with irrigation investigations of the United States Department of Agriculture. The moisture equivalents of the soils on which Mr. Harding worked varied from 14.9 to 29.3. His results show an average ratio of 1.17 in the surface foot of soil, of 1.16 in the upper 2 feet, and of 1.08 in the upper 5 feet. Considering the wide range of soils studied and the many variations in other conditions, these ratios agree very well with the ones above presented.

the difficulty in wetting these compact impervious soils. This high ratio may also be the result of difference in volume weight between the soil in the field and in the perforated cup of the centrifuge.[1] The probable error of a single comparison in the first group is ±0.06 for both foot sections; for the second group ±0.12 in the first foot and ±0.04 in the second; for the third group it is ±0.11.

TABLE X.—*Comparison of the maximum quantities of water contained after irrigation by the first- and second-foot sections of soil in place with their moisture equivalents*

SOILS HEAVILY IRRIGATED

Name of tract.	First foot of soil.				Second foot of soil.			
	Moisture equivalent.		Maximum quantity of water after irrigation (inches per foot of soil).	Ratio, moisture equivalent to maximum quantity after irrigation.	Moisture equivalent.		Maximum quantity of water after irrigation (inches per foot of soil).	Ratio, moisture equivalent to maximum quantity after irrigation.
	Per cent.	Inches per foot of soil.			Per cent.	Inches per foot of soil.		
Wigno	24.18	3.18	2.62	1.21	24.45	3.22	2.73	1.18
Griffes	20.64	2.98	2.58	1.15	21.22	3.06	2.57	1.19
Hundy	28.63	4.48	3.50	1.28	26.92	4.20	3.62	1.16
Hofhenke	22.12	*a* 3.45	3.27	1.05	22.12	3.45	3.29	1.05
Hughson	22.10	3.21	2.87	1.12	21.70	3.15	2.94	1.07
Huartson	21.46	3.38	2.92	1.16	22.10	3.48	2.90	1.20
O'Hair	23.87	3.18	3.14	1.01	21.70	2.89	2.51	1.15
Geer	24.75	3.78	3.67	1.03	26.71	4.08	3.86	1.06
University farm	23.88	3.67	3.25	1.13	21.90	3.37	3.10	1.09
Average	23.52	3.48	3.09	1.13±0.02	23.20	3.43	3.06	1.12±0.02

SOILS MODERATELY IRRIGATED

Guile	24.18	4.07	4.00	1.02	28.74	4.83	3.34	1.44
Jackson-Woodard	30.31	4.58	4.04	1.13	31.65	4.79	3.52	1.36
Beck	25.68	4.07	2.97	1.37	25.18	3.99	2.84	1.40
Wright	27.72	4.98	3.83	1.30	27.54	4.96	3.76	1.32
Average	26.97	4.42	3.71	1.20±0.06	28.28	4.64	3.36	1.38±0.02
Purdy	21.02	4.14	3.03	1.37	The impervious nature of these soils prevented the irrigation water from wetting the second foot; therefore comparisons are not made.			
Tuttle	19.30	4.03	3.09	1.34				
Willows' experimental tract, plots 3 and 4	19.79	4.14	2.40	1.72				
Willows' experimental tract, plots 6 and 7	22.59	4.75	2.97	1.60				
Willows' experimental tract, plots 11 and 12	20.70	4.35	2.91	1.50				
Average	20.66	4.28	2.88	1.48±0.05				

a Moisture-equivalent sample for first foot determined on soil from second foot.

The comparisons made in Table X suggest that the moisture equivalent may be made a means of judging the maximum capillary capacity

[1] That the volume weights of these clay soils are higher in the field than in the ordinary air-dry condition in the laboratory has been determined beyond reasonable doubt. If the same relative condition exists between the volume weights in the field and in the perforated cups of the centrifuge the increase of the ratio with increase of fineness of soil would be readily accounted for. However, volume-weight determinations were not made upon the soils after rotation in the centrifuge; nor have any such determinations been made, so far as known to the writer.

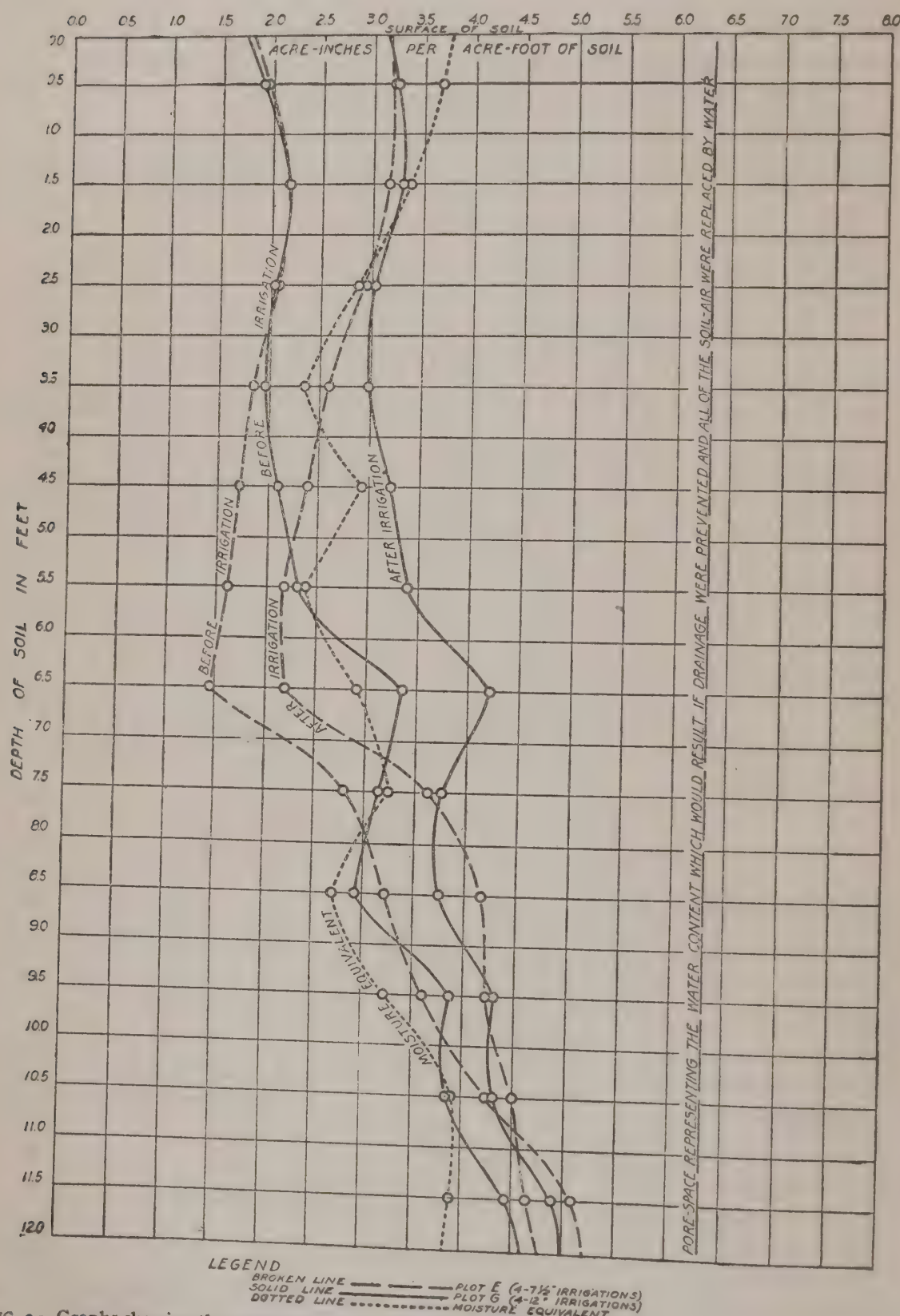

FIG. 9.—Graphs showing the comparison of water content before and after irrigation, moisture equivalent, and pore space of soils of plots E and G, Davis, Cal. Each water-content curve is the average of 28 borings.

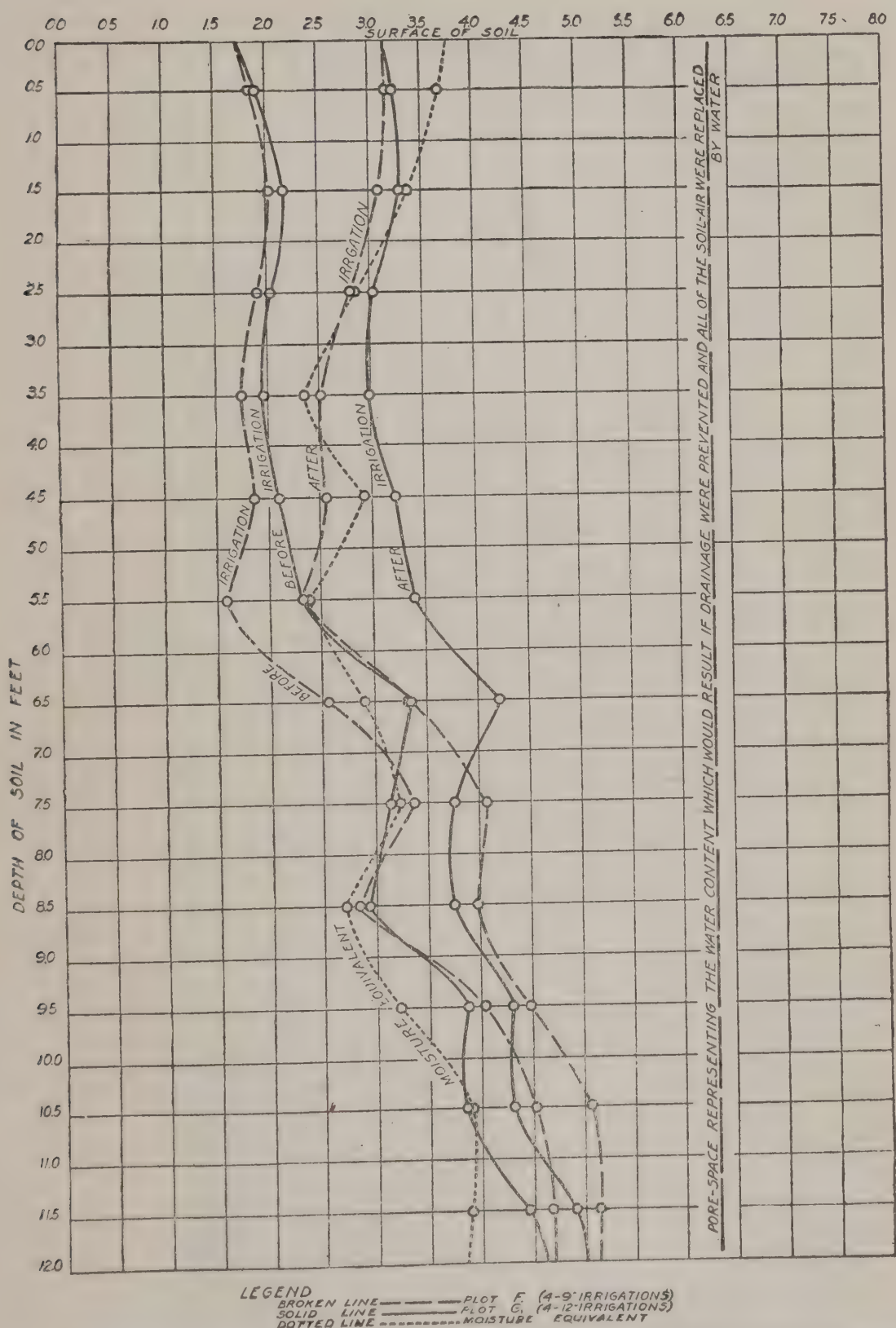

FIG. 10.—Graphs showing the comparison of water content before and after irrigation, moisture equivalent, and pore space of soils of plots F and G, Davis, Cal. Each water-content curve is the average of 28 borings.

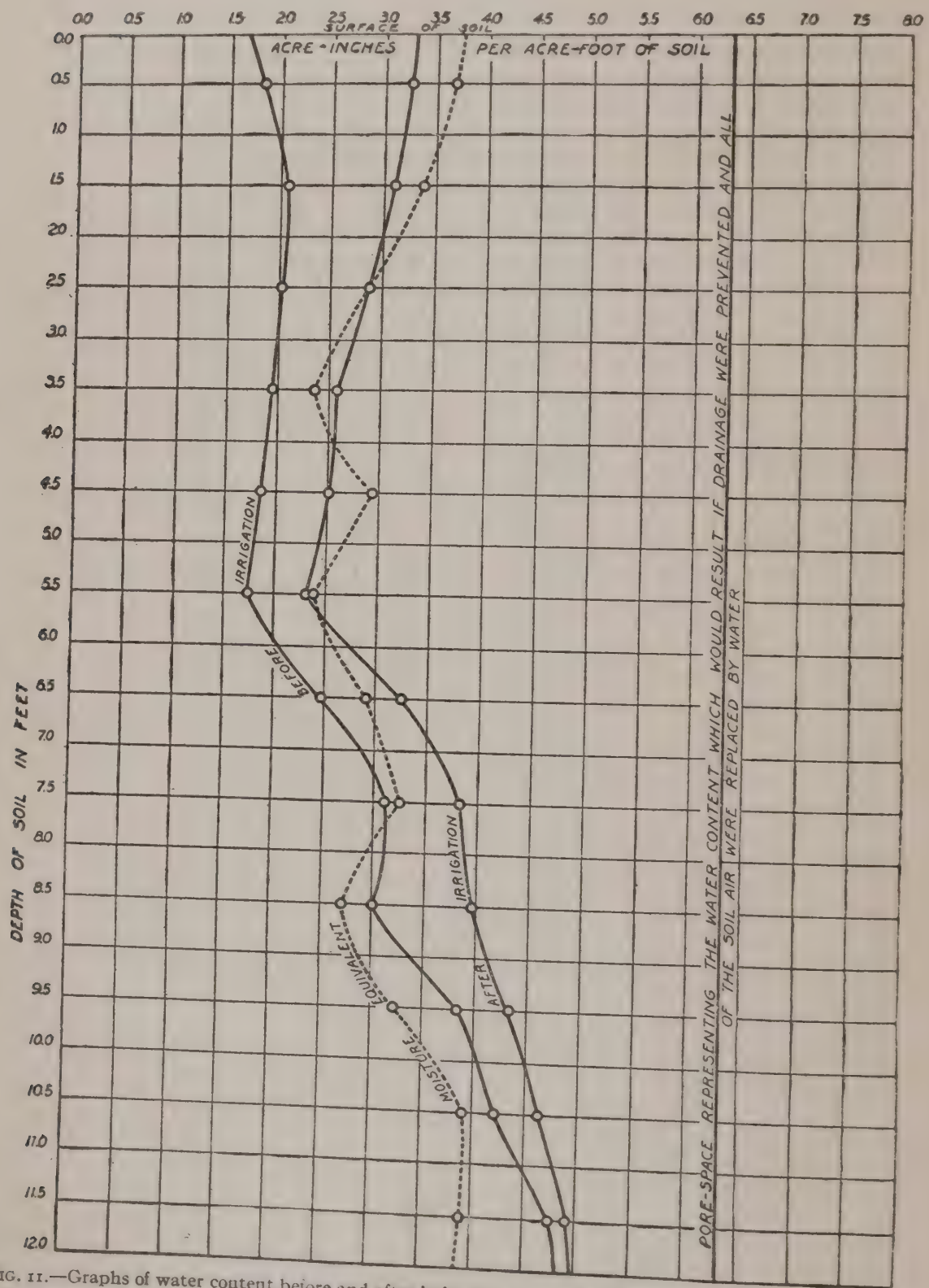

FIG. 11.—Graphs of water content before and after irrigation, moisture equivalent, and pore space of soils. Each water-content current is the average of 6 plots, B to G, for 1913, 1914, and 1915, and the average of 147 borings.

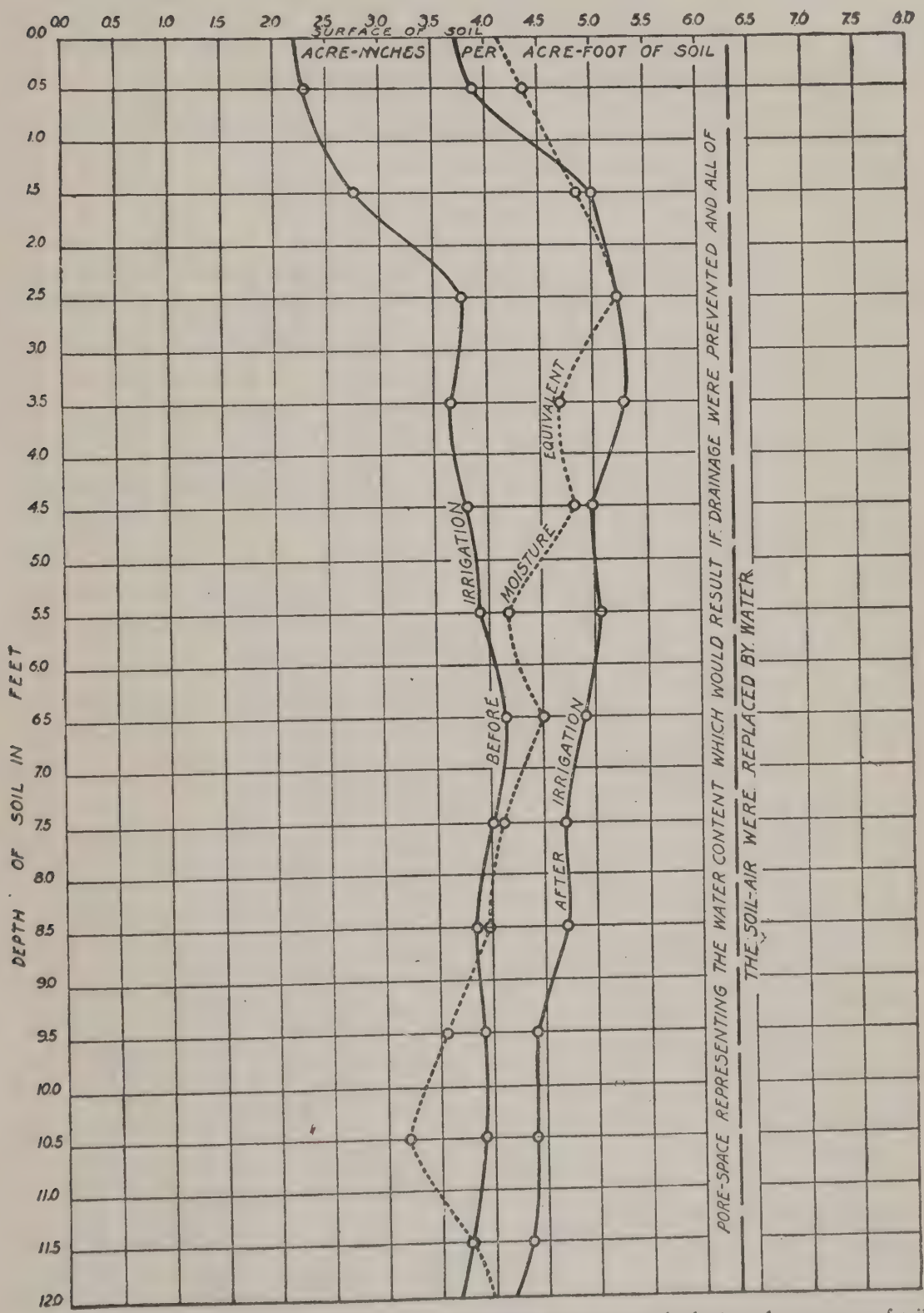

FIG. 12.—Graphs of water content before and after irrigation, moisture equivalent, and pore space of soils. Each water-content curve is the average of 28 borings on plot H (four 15-inch irrigations) for 1913, 1914, and 1915.

of soils in place. Though definite conclusions from so few correlations are not warranted, it seems that the moisture equivalent represents more nearly the maximum capillary capacity of the soil in place than do the ordinary laboratory determinations upon the disturbed soil, both in point of accuracy and of absolute value.

PART II.—A NEW METHOD OF DETERMINING THE VOLUME WEIGHTS OF SOILS

RUBBER-TUBE METHOD

In order to ascertain accurately by means of moisture determinations the volume of irrigation water which a given volume of soil absorbs and retains, it is obviously necessary to know with a fair degree of accuracy the volume weight of the soil in place. It was believed early in these studies and has since been verified that the ordinary method of determining volume weight of samples of disturbed soil could not be relied on. The use of an iron cylinder to be driven into the soil for determining its volume weight in place was considered. It was concluded that this method was unsatisfactory because of the tendency of the soil below the cylinder to become compacted and thus be driven ahead of, instead of into, the cylinder, and because of the time and expense involved in taking very many samples to the depths which must be considered in the soils of the arid regions. The first objection applies especially to the use of cylinders of small diameter, such as the King soil tube, and the second to larger tubes, with which the first objection may be measurably overcome.

The need of devising a new method which would overcome these objections seemed sufficiently urgent to warrant attention. The soil samples used for making moisture determinations were secured by the use of a 2-inch auger of the post-hole type. The diameter of the tip or cutting edge of the bowl of the auger used was slightly greater than that of the base, a fact which suggested that practically no displacement of soil by lateral thrust or compacting ahead of the auger would be caused by boring.

Upon the basis of these assumptions it was necessary only to devise a means of accurately measuring the volume of the hole made by a 6-foot boring in order to get a satisfactory measure of the volume weight of the soil, since the total amount of soil taken from the hole 2 inches in diameter and 6 feet deep could very conveniently be taken to the laboratory, dried, and weighed. Various methods of measuring the volume of the auger hole were considered. It was attempted to measure the diameter of the hole at different depths by means of calipers and thus compute the volume, but this proved unsatisfactory. Finally it was conceived that an accurate measurement of the volume could be obtained by inserting a very thin-walled elastic rubber tube into the auger hole

and filling it with water from a graduated cylinder. A special rubber tube having a diameter of 2¼ inches and a length of 6½ feet was secured and determinations were made of the volume weight as follows: Borings were made with the 2-inch auger above described to a depth of 1 foot, the soil being placed on an oilcloth and then into a suitable bag. The closed spherical end of the rubber was then forced into the hole upon the rounded end of a pole 1½ inches in diameter. Water was poured from a full 1,000-c. c. graduate cylinder into the tube until it was filled to a point flush with the surface of the soil.[1]

The water was then drawn out of the tube by means of a small pump, after which the tube was taken out of the hole and dried. Then the borings were continued to a depth of 2 feet and the volume of the hole again determined, the volume of the section from 1 to 2 feet being obtained by difference. This process was continued until the six upper 1-foot sections had been studied. The materials used are shown in Plate 1, A. The soils were oven dried and weighed. The volume of the rubber tube, 200 c. c., was taken into account in the determination of the total volume occupied by the undisturbed soil.

Laboratory volume-weight determinations were made upon the disturbed soil as follows: Brass tubes 2 inches in diameter and 10 inches long were filled with thoroughly pulverized air-dry soil on the Bowman compactor. The weight of the soil was corrected for hygroscopic moisture, and the volume of the tube was computed and also determined by filling it with water.

The volume weights of the upper 6 feet observed by the two methods are set forth in Table XI, in the last column of which the percentage decrease in volume weight caused by disturbing the soil is given. These percentages show great variation, which was not unexpected. The volume weight of the clay soil of the Willows experimental tract was decreased nearly 23 per cent by being disturbed, while that of Wigno tract (fine sandy loam) was increased 15 per cent. The most striking factor brought out by the study of the volume weight of the soil in place as presented in Table XI is the fact that the coarse-textured soils have in general much lower volume weights than the fine-textured ones, a relation just the reverse of that which is generally believed to exist between texture and volume weight.

[1] In using a 6-foot rubber tube for the first, second, and third foot sections, some difficulty was experienced in deterimining this point accurately. This difficulty could be overcome by providing several tubes of different lengths. For example, if it is desired to make a volume-weight determination of each foot section to a depth of 6 feet, the first tube used should be about 16 inches in length, the second 28, the third 40, and so on.

TABLE XI.—*Volume weights of different soils as determined (1) upon disturbed soil by the ordinary laboratory methods, and (2) upon soil in place by the rubber-tube method*

Name and location of tract.	Volume weight.		Percentage decrease in volume weight caused by disturbing the soil.
	By laboratory method upon disturbed soil.	By rubber-tube method with soil in place.	
University Farm, Davis	1.200	1.280±	6.3
Hughson, Woodland	1.220	1.210±0.015	−1.0
Jackson–Woodward, Woodland	1.135	1.257± .007	8.8
Guile, Woodland	1.185	1.398± .002	15.2
Purdy, Willows	1.340	1.642± .011	18.5
Tuttle, Willows	1.380	1.741± .019	20.8
Willows experimental tract, Willows	1.350	1.750± .010	22.9
Wigno, Los Molinos	1.280	1.112± .019	−15.0
Bundy, Los Molinos	1.200	1.289± .001	6.8
Geer, Los Molinos	1.350	1.272± .013	− 6.0

OTHER METHODS USED

In order to check the above method, further studies were conducted cooperatively with Prof. Charles F. Shaw, (3) of the Division of Soil Technology, University of California, the work being done chiefly by the paraffin-immersion method employed by him at the Pennsylvania Agricultural Experiment Station.[1] Further volume-weight determinations were also made by use of an iron cylinder 6 inches in diameter. The procedure in the paraffin-immersion method is outlined below: A hole, 3 feet wide and 6 feet long, was excavated to a depth of 5 feet by use of pick and shovel. At one end steps were made in the soil for convenience in getting into and out of the hole. One side of the excavation was carefully smoothed and plumbed. From this side two sections of soil 1 foot apart and of approximately 1 foot in cross section were excavated, thus leaving a vertical column 1 foot square and 5 feet high, having three sides exposed, as represented in figure 13. The top of this column was carefully smoothed by means of a spatula, putty knife, and trowel. The fourth side of the column was cut from its base to a depth of 6 inches with a spatula; and at the same depth below the surface of the column the spatula was inserted horizontally from the three exposed sides and thus a ½ cubic-foot sample, 1 foot square and ½ foot long, representing approximately the upper 6 inches of soil, was severed from the 5-foot column. The sample was placed upon the platform, as shown in Plate 1, B, cut into four cubes of approximately equal size, the best three of which were used in determining the volume weight.

[1] For a description of the method proposed by Prof. Shaw, see BROWN, B. E., MACINTIRE, W. H., and CREE, W. F. COMPARATIVE PHYSICAL AND CHEMICAL STUDIES OF FIVE PLATS, TREATED INDEPENDENTLY FOR TWENTY-EIGHT YEARS. *In* Penn. Agr. Exp. Sta. Ann. Rpt., 1909–10, p. 96–97. 1910.

Each sample was placed on a weighed wire-mesh support, weighed upon a solution balance, dipped into warm paraffin until fully coated (usually three or four times), reweighed, and placed into a large desiccator of known volume.[1]

The desiccator containing the soil, paraffin coating, and wire-mesh support was filled with water from a 1,000-c. c. graduate cylinder and the volume of the soil, paraffin, etc., determined by displacement. From the known weights of the paraffin and wire-mesh support their volumes were computed, using 0.9 as the density of paraffin and 8.0 as the density of the wire. After the volume measurement the cubes of soil were broken and a 200-gm. sample taken for the determination of the moisture content. The results are presented in the last column of Table XII.[2]

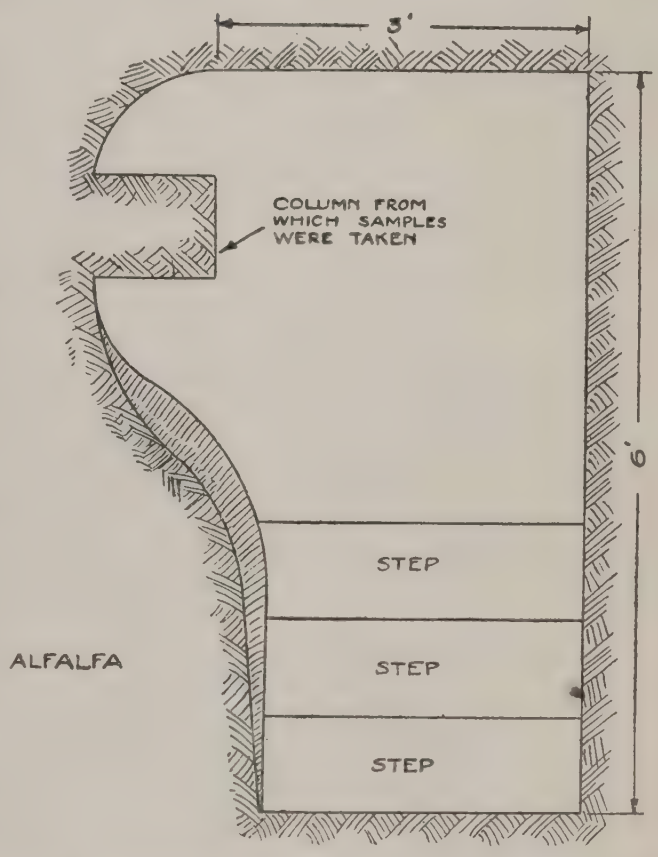

Fig. 13.—Diagram showing plan of excavation for the determination of the volume weight of soil by means of the paraffin-immersion method.

The results presented in column 3 of Table XII were obtaind by the use of a specially prepared iron cylinder, having a diameter of 6 inches, which could be driven into soils of ordinary compactness only with great difficulty. It was therefore not attempted to drive it into this very compact clay; but the top of a column of soil having a circular cross section of about 8 inches in diameter was carefully smoothed and leveled, after which two spatulas were inserted horizontally into the column. The iron cylinder was placed vertically upon the column, as indicated in figure 14. The column was trimmed very carefully with a trowel until its diameter was slightly greater than that of the cylinder. The weight of the cylinder, aided by very light tapping, caused it to move slowly down over the soil column.

[1] The cover of the desiccator used has a 1-inch diameter opening in its center, thus making it possible to measure the desiccator volume accurately.

[2] The probable errors were determined by use of Peter's Formula $Rm = \frac{0.845 \Sigma V}{n\sqrt{n-1}}$. Where Rm=the probable error of the arithmetic mean of n determinations, and ΣV=the sum of the differences between the arithmetic mean and each determination. In this case $n=3$. It is recognized that where n is so small the formula is not strictly accurate.

TABLE XII.—*Volume weights of Tehama clay, Willows experimental tract, Willows, as determined (1) upon the disturbed soil by the ordinary laboratory method, (2) upon the soil in place, (a) by the rubber-tube method, (b) by the iron-cylinder method, and (c) by the paraffin-immersion method*

Depth of soil.	Laboratory method on disturbed soil.[a]	Rubber-tube method with soil in place.[b]	Iron-cylinder method with soil in place.[c]	Paraffin-immersion method with soil in place.
2 to 7 inches	.....	.....	1.632	1.671±0.004
8 to 13 inches	.....	.....	1.721	1.702± .008
15 to 20 inches	.....	.....	1.781	1.784± .007
22 to 27 inches	.....	.....	1.750	1.802± .002
28 to 33 inches	1.380±0.007	.....	1.828	1.802± .007
35 to 40 inches	.....	.....	1.780	1.792± .000
42 to 47 inches	.....	.....	1.732	1.757± .012
48 to 53 inches	.....	.....	1.625	1.747± .010
54 to 60 inches	1.320± .004	.....	.....	1.541± .028
Average, 0 to 60 inches	1.350± .008	1.744±0.010	1.731	1.733± .035

[a] Laboratory determinations were made upon composite samples from only two depths: (1) from 0 to 2.5 feet, and (2) from 2.5 to 5 feet. The results given are averages of 12 determinations from each depth.
[b] Only one depth considered in rubber-tube method, 0 to 5 feet.
[c] Depths given for iron-cylinder method are almost but not strictly accurate. Total depth covered by eight samples in this method, 58 inches. Lack of rigid accuracy in depths due to slight but unavoidable loss of soil in preparing each 6-inch section for the cylinder.

As soon as it reached the spatula blades, the 6-inch column within the cylinder was severed from its base, and the end was carefully smoothed

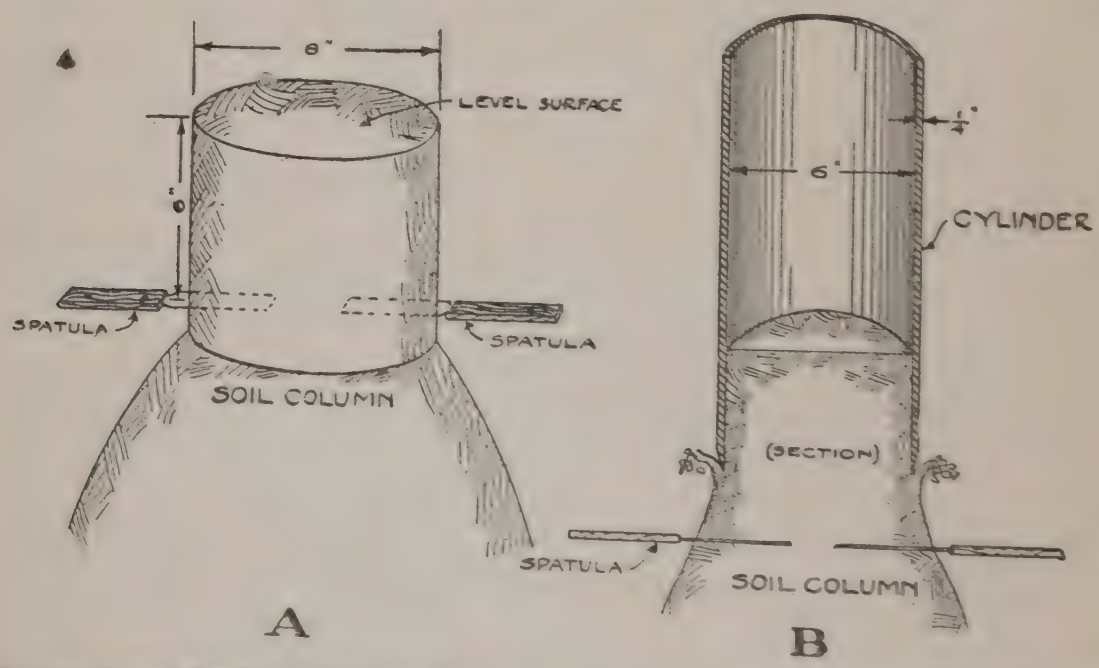

FIG. 14.—Diagram showing method used for the determination of the volume weight of soil by the use of an iron cylinder: *A*, column of soil ready for determination; *B*, cylinder being placed over the column of soil.

flush with the cylinder, after which the soil was taken out, weighed, and sampled for a moisture determination. The process was continued until eight samples had been taken. These covered a total depth of

not quite 5 feet, since some soil was lost in smoothing the column. Compacting in the cylinder was very largely, if not entirely, overcome. Measurements of the length of the column in the cylinder gave results varying from 6⅛ to 5⅞ inches. These variations are believed to be due to the difficulty found in inserting the spatula at the 6-inch point without deviating from the horizontal plane rather than to any change in structure of the soil.

Only one sample was taken at each depth; consequently probable-error calculations can not be made. Very gratifying agreement exists between the results obtained by use of the iron cylinder and those obtained by the paraffin-immersion method, as presented in Table XII. Especially is this true of the averages for the upper 5 feet, wherein the difference is entirely insignificant. Moreover, the results obtained by the paraffin-immersion method and the iron-tube method, which were secured in March, 1916, confirmed beyond doubt the correctness of the results obtained by the rubber-tube method in October, 1915, at the end of the irrigation season.

SUMMARY

(1) This paper reports some observations upon the capacities of certain soils under different conditions to retain water and also develops a new method of determining the volume weight of soils in place, representing some phases of a six-year study of the economical duty of water for alfalfa in Sacramento Valley.

A relation between the depth of water necessary to add a given percentage of moisture to a certain depth of soil of given volume weight is expressed mathematically and graphically.

The observations of capacity of soils to retain water are based on 9,584 moisture determinations in the upper 6 feet of soil, 672 in the depth from 7 to 9 feet, and 192 in the tenth to twelfth foot sections, making 10,448 in all.

Volume-weight determinations upon which the pore-space values largely depend and by which the percentages of water were converted to inches of water per foot of soil were made upon the soils in place to a depth of 6 feet.

(2) The observations indicate that percentages of pore space which are filled by the water that a soil holds immediately after irrigation increases with the increase in fineness of soil texture. Variations from 40 per cent in silt-loam soils having fine sandy-loam subsoils, 51 per cent in the silt loams, 58 per cent in the clay loams, to 66 per cent in the clay soils have been noted.

(3) The ratio of the maximum capillary capacities of soils, as determined in a 10-inch tube in the laboratory, to that of the same soils observed in the field after irrigation varied from 1.78 ± 0.06 to 1.98 ± 0.14.

(4) Correlations between the moisture equivalent and the maximum amounts of water found after irrigation show a gratifying agreement and suggest that the moisture equivalent might be made a basis of judging maximum capillary capacities.

(5) A new method of determining volume weight of soil in place which is simple of manipulation and inexpensive is described. The results of the new method of determining the volume weight of clay-loam soil, as checked by a paraffin-immersion method first used by Prof. Charles F. Shaw and by the use of an iron tube, were subject to an error of less than 1 per cent.

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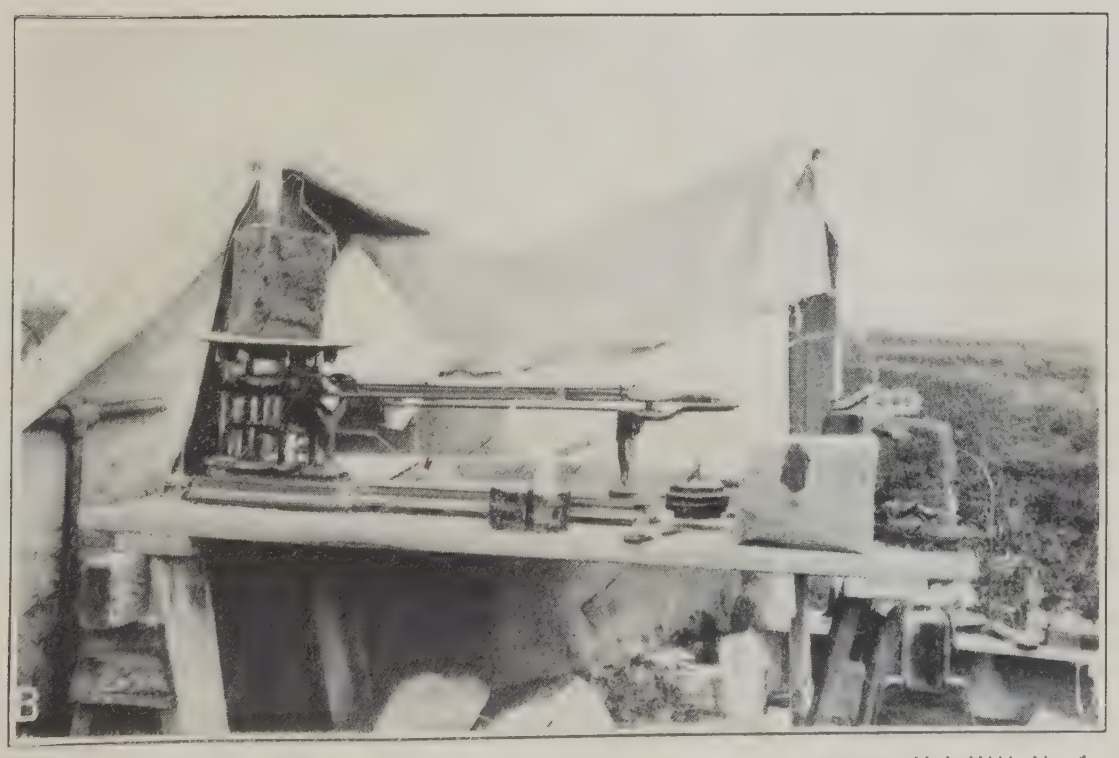
PLATE I

A.—Apparatus used for the determination of the volume weight of soils in place by the rubber-tube method.

B.—Apparatus used for the determination of the volume weight of soils in place by the paraffin- immersion method.

A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SOME STONEFLIES INJURIOUS TO VEGETATION

By E. J. Newcomer

Scientific Assistant, Deciduous Fruit Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

Stoneflies (Plecoptera) are not ordinarily classed among the insects injurious to plant life. Indeed, a study of the mouth parts of several species has shown them to be more or less rudimentary,[1] so that, while they are of the biting type, they are not capable of being used to inflict injury upon plant growth. John B. Smith says[2] that adult stoneflies do no feeding upon living plants, so far as known. The writer has been much interested, therefore, in studying the habits of several of the western species of the genus Taeniopteryx (*Taeniopteryx pacifica* Banks, *T. pallida* Banks, and *T. nigripennis* Banks), which are equipped with well-developed mouth parts, and which feed upon the buds and leaves of plants. One species in particular, *T. pacifica*, has proved to be of considerable economic importance in the Wenatchee Valley, in central Washington. While it has not been possible to make detailed life-history studies, or to work out satisfactory control methods, it has been thought advisable to publish a short paper on the subject, owing to the unusual habits of these species.

OCCURRENCE

When the writer arrived at Wenatchee, Wash., in May, 1914, he was shown injuries to foliage and fruit which had been caused by an insect known locally as the "salmon fly." Unfortunately the insects had all disappeared. However, the writer recalled an early experience in the Yellowstone Park where insects known as "salmon flies" were plucked from one's clothing or from the adjacent foliage and used as bait in catching salmon trout from the Yellowstone River. These insects were a species of stonefly, and the descriptions given the writer of the insect at Wenatchee indicated that it was also a stonefly. During the following two years (1915 and 1916) ample opportunity was afforded for studying the habits of the salmon fly, which proved to be a stonefly, and which has been identified by Mr. Nathan Banks, formerly of the Bureau of Entomology, as *Taeniopteryx pacifica*.

[1] Smith, Lucy W. THE BIOLOGY OF PERLA IMMARGINATA SAY. *In* Ann. Ent. Soc. Amer., v. 6, no. 2, p. 203–212, illus., pl. 23. 1913.

[2] Smith, John B. INSECTS OF NEW JERSEY. *In* Ann. Rpt. N. J. State Mus., 1909, p. 15–880, illus. 1910.

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In 1915 the stoneflies appeared early in March, becoming common by the middle of the month. They were observed flying about on warm days, and examinations of the various fruit trees showed that the flies were resting on the twigs and branches in some numbers. In 1916 the flies did not appear until March 22, as the season was late, but on this date, which was the first warm spring day, they were very numerous. At this time most of the fruit buds were beginning to swell. The flies were abundant during both seasons for three or four weeks, after which they disappeared. The distribution of the species is not well known. The type specimens came from Pullman, Wash., which is 160 miles southeast of Wenatchee, and it is probable that this insect occurs throughout the arid region of the Northwest, wherever there are streams of sufficient size for the nymphs.

HABITS

The economic importance of this stonefly lies in its habit of eating the foliage and of biting into the buds of fruit trees. When the flies first appear the fruit buds are beginning to push out, and the flies eat large holes in them, frequently destroying them entirely (Pl. 2, B). Even where the injury is not so severe the blossoms and leaves developing from these buds are deformed and ragged (Pl. 2, A). The ovary of the blossom is very often injured, resulting in deformed fruit. Later the insects feed on the calyces and corollas of the blossoms, on the young fruit (Pl. 3, B), and on the tender foliage (Pl. 3, A,). Apricots, peaches, and plums are most seriously injured. Their buds are soft and tender, and the stoneflies have no difficulty in feeding on them. Cherries are not so noticeably injured, the buds being harder and the young foliage being sticky. The damage to apples and pears is negligible, as their buds are tougher and they blossom later.

The insects may be found commonly lying lengthwise along the twigs (Pl. 2, C), and sometimes on the larger branches. Frequently, by jarring the branches, hundreds of them will be dislodged, and they will drop to the ground or fly awkwardly to other trees. They do not appear to feed when the weather is cold or cloudy, but during the warmer parts of sunny spring days they are quite actively moving about and feeding on the buds or young foliage. Often they will be found with their heads half buried in the holes they have eaten in the buds, their long, filamentous antennæ waving continually. If slightly disturbed, they will stop feeding and remain motionless, or slide around to the other side of the twig. Both sexes have been found in the trees, and mating has been observed to take place here. It is evident that this habit of feeding in fruit trees is an acquired one, since the early stages of the insect are passed in the natural streams, and it was undoubtedly abundant before fruit trees were ever planted in the valley. The native vegetation, especially that along the streams, was carefully examined,

and the stonefly was observed feeding to some extent on the leaves of the wild rose, on the leaves and catkins of the willows (*Salix* spp.), and on the leaves of the wild cherry (*Prunus emarginata* and *P. demissa*) and alder (*Alnus tenuifolia*), and also on the cultivated elm (*Ulmus americana*). The insect seems to prefer the rosaceous plants, although it does not confine its feeding to them.

The injury caused by this stonefly was quite noticeable, especially in the lower part of the Wenatchee Valley, known as the Rock Island district, where there are extensive orchards near the Columbia River. Many growers here said that it was very seriously damaging their apricots and peaches, necessitating the discarding of much of the fruit.

CONTROL

Owing to other work, it was not possible to carry out any extensive experiments in the control of the stonefly. It was noted in 1915, however, that plum trees which had been sprayed with crude-oil emulsion and nicotine sulphate for aphids were not as badly injured as those not sprayed. Several growers reported spraying their trees with nicotine sulphate and soap, with varying success. On April 3, 1916, an apricot orchard was examined, part of which had been sprayed about April 1 with lead arsenate at the strength ordinarily used for the codling moth on apples (2 pounds of lead arsenate to 50 gallons of water). At this time the buds were beginning to show green. A number of buds were examined and counted on both sprayed and unsprayed trees. On the latter trees 60 per cent of the buds were injured, while on the former only 24 per cent were injured, and it is probable that much of this latter injury was done before the trees were sprayed, as the flies had been numerous for over a week. It seems probable that in order to protect the trees completely two applications of spray would be necessary, as the buds are developing rapidly at this time. The first application would naturally be put on as soon as the flies appear, and the second either just before blossoming or just as the petals are falling.

LIFE HISTORY

At the time the stoneflies were abundant several of the smaller streams flowing into the Columbia River near Wenatchee were examined, but no emerging flies were found. The shores of the Columbia River, which at Wenatchee is a large, swiftly flowing stream about one-fourth of a mile wide, were then examined, and the flies were found emerging in large numbers. Thousands of cast nymphal skins (Pl. 3, C) were strewn along the shore from the water's edge to 10 or 15 feet above it. Hundreds of crippled flies were scrambling about over the rocks, but few perfect ones were seen, as these evidently fly away as soon as their wings are sufficiently dried. In the shallow water under the larger stones the nymphs were found, most of them just ready to emerge.

As mating was observed in the orchards, and as the bodies of most of the female stoneflies taken here contained eggs, it is evident that the females must return to the river after feeding to deposit their eggs. These eggs hatch in the water, and the nymphs develop slowly, the adults emerging in the spring as soon as the weather becomes warm. Whether the nymphs complete their development in a single year, or require two years or more, is not known.

DESCRIPTIONS OF STAGES

EGG.—Oviposition was not observed; nor were eggs found in the water; but eggs dissected from the body of the female were approximately spherical and about 0.2 mm. in diameter.

NYMPH (Pl. 3, C).—The full-grown nymph is about 10 mm. long, exclusive of the antennæ and cerci. The prothorax and abdomen are dull, dark brown, the head is a lighter brown, the eyes black, and the mesothorax, metathorax, and all appendages a very light brown. The filamentous antennæ are about 6.5 mm. long and the abdominal cerci about 8 mm. long. The legs are fringed with long hairs. No gills were present on the specimens collected, which were just ready to emerge and may have lost them.

ADULT (Pl. 3, C; Pl. 4, A).—The original description of the adult is given herewith:

Head dull black; antennæ brown; prothorax dull black, anterior margin, and usually the lateral margins, narrowly reddish; base of mesothorax reddish, rest of body black; legs yellow-brown, knees rather darker. Wings dull hyaline, without marks, or an indistinct cloud near the middle, hind pair hyaline, veins brown. Prothorax rather broader than long, a transverse sulcus in front, on the disk are scattered small flat tubercles or scars; second joint of tarsi as long as first, tips of tibiæ with a pair of minute spines; ventral plate of the female is nearly semicircular. Wings long, slender, subcostal with several cross-veins to margin near tip, and a few near base, radial sector with but one fork beyond the anastomosis, the vein from the discal cell arises near the radial sector, pterostigmatic region long, with but one cross-vein.

Length to tip of wings, 12 mm.

Pullman, Wash., April (C. V. Piper, R. W. Doane).[1]

DESCRIPTION OF MOUTH PARTS

Since the structure of the mouth parts determines the economic importance of an insect of this type, a detailed description of these will not be out of place here.

ADULT (PL. 4)

LABRUM.—Subquadrate, somewhat broader than long, sparsely covered with setæ; sides convex and heavily chitinized; distal margin slightly concave.

MANDIBLES.—Subtriangular, the outer margin slightly rounded, the cutting edge provided with a series of strongly chitinized teeth, which are unlike on the two mandibles. The right mandible has a sharply pointed tooth at the outer edge, behind which are three bladelike teeth and a protruding grinding edge. The left mandible has a row of three sharply pointed teeth, behind which are two bladelike teeth, the second being serrate, and a grinding edge.

MAXILLÆ.—Of the usual biting type. Galea digitiform, lacinia tapering, provided distally with two chitinous teeth, one longer and heavier than the other, and with a row of spines along the inner margin. Palpus four-jointed, covered with many setæ.

[1] BANKS, Nathan. NEW GENERA AND SPECIES OF NEARCTIC NEUROPTEROID INSECTS. *In* Trans. Amer. Ent. Soc., v. 26, no. 3, p. 244. 1900.

LABIUM.—Rather simple. Glossæ small and tapering; paraglossæ larger, thumb-like; both provided with long and short spines. Palpi three-jointed; a distinct tactile surface on the inner side of the tip, covered with short spines.

NYMPH

The nymphal mouth parts are practically identical with those of the adult, with the exception of the mandibles. The teeth of the latter, particularly the left mandible, are somewhat blunter, and the grinding edge of the left mandible is provided with a row of stout spines.

OTHER SPECIES

On May 31, 1915, a trip was taken into the mountains back of Wenatchee, and there, in the pine woods at an elevation of 3,000 feet, two distinct species of stoneflies were found feeding on the native vegetation along the banks of a swiftly flowing stream. These species have been identified by Mr. Banks as *Taeniopteryx nigripennis* and *T. pallida*. The former species was much the commonest, is smaller than *T. pacifica*, being only 9 mm. in length, and is black. *T. pallida* is slightly larger, and brown, with light wings. The foliage along the stream had quite a ragged appearance, as these species were very numerous and feeding voraciously. The plants affected, in the order of the preference of the stoneflies for them, were the thimbleberry (*Rubus parviflorus*), alder (*Alnus tenuifolia*), willows (*Salix* spp.), wild rose (*Rosa* sp.), serviceberry (*Amelanchier* sp.), and maple (*Acer douglasii*).

The mouth parts of these species have been examined and found to be very similar to those of *T. pacifica*. They are, of course, smaller, but fully as well equipped for biting soft plant tissues.

On April 25, 1917, a single stonefly was observed at Salem, Oreg., feeding on young cherry leaves. This specimen was captured, and has been determined as *Taeniopteryx* sp. by Mr. A. N. Caudell, of the Bureau of Entomology. A search failed to reveal any more specimens.

One may conclude from these observations that a study of the habits of stoneflies in other parts of the country, particularly those of the genus Taeniopteryx, will bring to light other plant-feeding and, hence, potentially injurious species.

PLATE 2

Taeniopteryx pacifica:

A.—Young peach leaves and blossoms injured by stonefly.
B, C.—Adult stoneflies feeding on peach buds.

A
B
C

A
B
C

PLATE 3

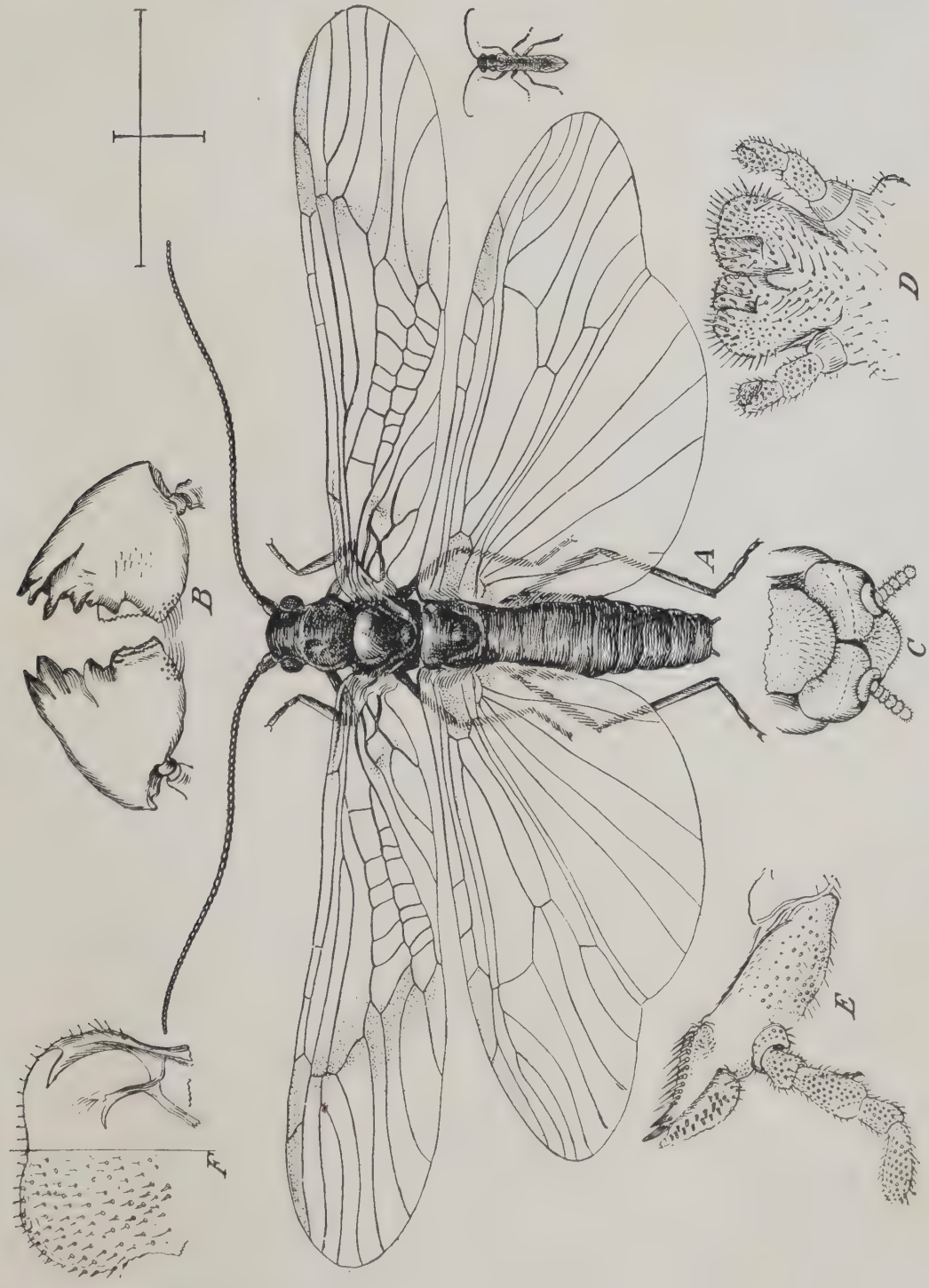
Taeniopteryx pacifica:

A.—Cherry foliage injured by stoneflies.
B.—Partly grown peaches, showing injuries caused by stoneflies.
C.—Nymph, cast nymphal skin, and adult stonefly.

PLATE 4

Taeniopteryx pacifica:

A.—Stonefly, much enlarged, and natural size.
B.—Mandibles, ventral view. Right mandible at reader's left.
C.—Ventral view of anal segment of female.
D.—Labium.
E.—Maxilla.
F.—Labrum. Dorsal view at left, ventral view at right.


A
B
C
D
E
F

BASAL KATABOLISM OF CATTLE AND OTHER SPECIES

By Henry Prentiss Armsby, *Director*, J. August Fries, *Assistant Director*, and Winfred Waite Braman, *Associate, Institute of Animal Nutrition of The Pennsylvania State College*

COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF ANIMAL INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE INSTITUTE OF ANIMAL NUTRITION OF THE PENNSYLVANIA STATE COLLEGE

INTRODUCTION

The term "basal katabolism" has become generally accepted as a convenient designation for that portion of the katabolism due to the fundamental vital processes as distinguished, on the one hand, from that arising from external muscular activities and, on the other hand, from that caused by the ingestion of food. It is the katabolism of the animal in a state of complete muscular rest and with the processes of digestion and resorption suspended. It is the irreducible minimum of metabolic activity consistent with a particular condition of the body.

The basal katabolism in this strict sense is to some degree an ideal conception, although a close approach to the ideal may be observed in the postresorptive condition during short periods of complete muscular relaxation. Basal katabolism as thus defined is, from a slightly different point of view, an expression of the minimum food requirement of the organism. Any surplus above this minimum may either be utilized for muscular activity or give rise to a storage of matter and energy.

As regards the necessary food supply, however, a knowledge of the basal katabolism in the strict sense is of limited utility. A state of complete muscular inactivity can not be maintained for any considerable time, while even slight exertion augments the katabolism to a marked degree. In estimating the food requirements for the performance of a given amount of work by man or domestic animals, or for the storage of a specific quantity of protein and fat in the form of meat or milk, the base line is afforded, not by the katabolism during short periods of absolute rest but by the fasting katabolism of the individual under average conditions of living. Particularly is this true of the feeding of domestic animals for the production of human food. The dairy cow or the fattening steer must receive enough feed to supply its incidental daily activities as well as its minimum katabolism in a state of absolute rest before any is permanently available for manufacture into milk or meat. This amount, commonly spoken of as the maintenance requirement, is measured by the fasting katabolism under average conditions and may be called the 24-hour basal katabolism. Obviously, this is not as sharply defined a conception as is the basal katabolism in the narrower sense, but its practical importance is evident.

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METHODS OF EXPERIMENTATION

The determination of the fasting katabolism of animals such as Carnivora, man, or swine, having a comparatively simple apparatus, is largely a question of experimental technic. With ruminants the case is different. Their digestive apparatus is capacious and complicated, and contains at all times a relatively large amount of feed in process of digestion. It seems scarcely possible by any moderate period of fasting to reach a condition corresponding to the postresorptive state in man or Carnivora. The fasting katabolism may, however, be obtained indirectly by a comparison of the total metabolism on two different amounts of the same feed in the manner described in previous publications (*1, p. 33; 2, p. 282; 3, p. 53; 6, p. 460*).[1] For example, a steer receiving two different amounts of the same mixed ration gave the following results:

Item.	Dry matter eaten daily.	Daily heat production.
	Kgm.	*Calories.*
Period 2	9.146	16,511
Period 1	4.463	10,905
Difference	4.683	5,606
Heat increment per kilogram of dry matter	.....	1,197

Evidently, out of the total metabolism of 10,905 Calories in period 1, $1{,}197 \times 4.463 = 5{,}342$ Calories may be regarded as the heat production caused by the 4.463 kgm. of dry matter eaten, while the remainder, 5,563 Calories, is the basal katabolism.

Strictly speaking, the foregoing method of computation assumes that the heat production caused by the feed is a linear function of its amount. This can not be regarded as having been proved, but no distinct indications to the contrary have appeared within the range of our experiments, while Wood and Yule (*21, p. 239*) compute that in Kellner's respiration experiments on fattening cattle the gains expressed in terms of energy are proportional to the metabolizable energy supplied in excess of maintenance, which, of course, is equivalent to saying that the heat production is also a linear function of the feed supply.

Our investigations on the metabolism of cattle afford data for a number of computations of the basal katabolism. Full statements regarding the methods employed, the animals used, the feed consumed, etc., have already been published (*4, 5, 6, 7*). The designations of the experiments, animals, and periods in the following pages correspond with those in the publications cited.

In view of the very striking effect of standing in increasing the metabolism of cattle the basal katabolism per 24 hours has been computed

[1] Reference is made by number (italic) to "Literature cited," pp. 55–57.

separately from the observed rate of heat production during the intervals of lying and standing, respectively, and also for 12 hours standing and 12 hours lying per day, assumed as representing average conditions (Table I).

TABLE I.—*Computed 24-hour basal katabolism of cattle per head*

Feeding stuffs.	Experiment No.	Animal No.	Periods compared.	Basal katabolism.			Average live weight.	Approximate age.
				Lying 24 hours.	Standing 12 hours.	Standing 24 hours.		
				Cal.	*Cal.*	*Cal.*	*Kgm.*	*Months.*
Timothy hay	174	I	D–A	6,136	6,927	7,717	406	36
Red clover hay	179	I	1–2	2,992	6,502	7,006	533	48
Do	186	I	2a–1a	7,509	8,298	9,087	579	60
Do	186	I	2b–3b	8,157	8,828	9,499	571	60
Timothy hay	190	A	4–3	2,963	4,197	5,341	275	11
Do	190	B	4–3	1,979	3,445	3,487	192	13
Do	200,	A	4–3	5,395	5,845	6,277	403	23
Do	200	B	4–3	4,143	4,819	5,521	303	25
Do	207	A	4–3	4,489	5,105	5,695	511	35
Do	207	B	4–3	5,226	5,395	5,935	380	37
Alfalfa hay	208	D	1–2	2,449	2,525	2,602	167	9
Do	208	E	4–6	2,274	2,867	3,462	211	9
Alfalfa hay and mixed grain.	208	E	1–3	2,945	3,742	4,537	204	9
Alfalfa hay	208	C	4–6	2,696	3,759	4,841	282	9
Alfalfa hay and mixed grain.	208	C	2–3	3,209	4,370	5,532	264	9
Alfalfa hay	209	F	4–6	3,591	4,134	4,679	307	21
Alfalfa hay and mixed grain.	209	F	1–3	4,015	4,913	5,810	292	21
Maize stover	210	D	1–3	3,647	4,746	5,828	331	21
Mixed hay	211	D	1–5	5,124	6,601	8,066	444	33
Mixed hay and hominy feed.	211	D	3–2	4,298	5,921	7,531	451	33
Mixed hay	211	G	1–5	4,525	5,818	7,098	377	28
Mixed hay and maize meal.	211	G	3–2	4,577	5,446	6,403	378	28
Alfalfa hay	212	H	1–5	3,650	4,760	5,551	343	20
Alfalfa meal	212	H	2–6	2,545	3,314	4,142	339	20
Alfalfa hay	216	J	5–7	3,853	5,127	6,342	390	21
Alfalfa hay and starch	216	J	1–4	4,560	5,348	6,157	372	21
Alfalfa hay and mixed grain.	217	J	2–1	4,882	5,563	6,243	513	33
Do	217	J	3–4	6,474	7,544	8,618	649	33

In experiment 217 steer J was fattened previous to periods 3 and 4 with the result of greatly increasing his basal katabolism both per head and per unit of surface (*6*). The result in these two periods, therefore, does not seem to be comparable with the others and has been omitted in the following comparisons. Steer C had also been full-fed from birth for the production of baby beef, but was by no means so fat as the more mature steer J and has been included in the discussion.

The results of Table I are shown graphically in figures 1, 2, and 3 in which the crosses represent the individual results, the straight lines the mean katabolism computed in proportion to the live weight, and the curves the mean katabolism computed in proportion to the body surface as recorded in Table III. Both the tabulated results and the graphs show rather wide variations, perhaps due in part to the fact that the computation is one by difference. As would be expected, the basal katabolism increased in general with the size of the animal but with very considerable fluctuations. According to Rubner's surface law, the basal katabolism within the same species is approximately proportional to the two-thirds power of the live weight. To test the extent to which this was true in these experiments, the coefficient of correlation [1] of the basal katabolism with the live weight and with the two-thirds power of the live weight has been computed, with the results shown in Table II.

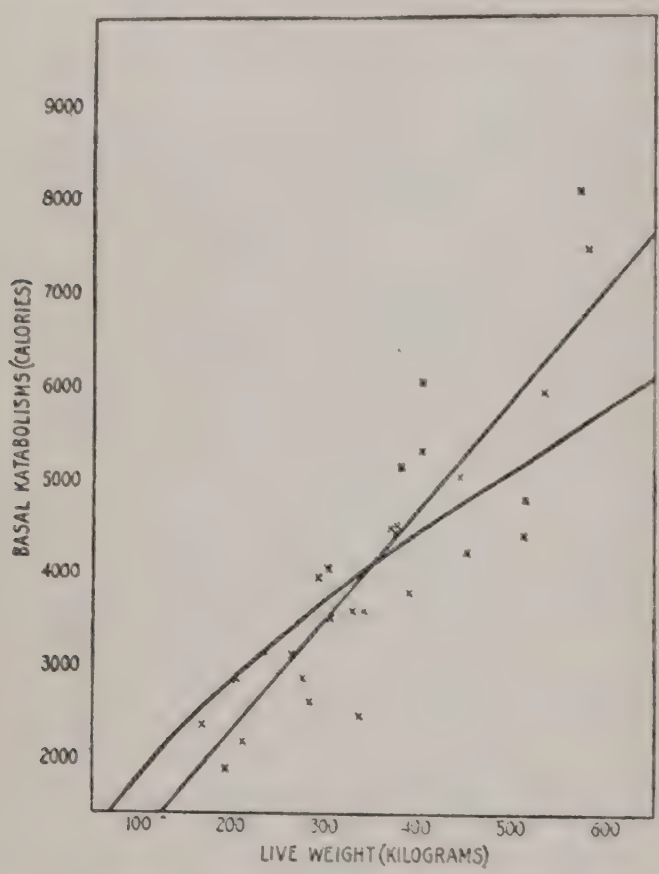

FIG. 1.—Graph of the basal katabolism of cattle per 24 hours' lying.

TABLE II.—*Coefficients of correlation*

	With live weight.	With two-thirds power of live weight.
Basal katabolism, lying 24 hours	0.8655±0.0326	0.9032±0.0239
Basal katabolism, standing 12 hours	.8733± .0308	.8710± .0313
Basal katabolism, standing 24 hours	.8548± .0350	.8250± .0415

Rather high coefficients were naturally to be expected, but the results fail to show any closer correlation with the two-thirds power of the weight than with the weight itself, a fact which is in harmony with Benedict's results upon man cited on a subsequent page.

[1] The statistical computations throughout this paper follow the methods described by C. B. Davenport in Chapter II of his "Statistical Methods with Special Reference to Biological Variation." (DAVENPORT, C. B. STATISTICAL METHODS, WITH SPECIAL REFERENCE TO BIOLOGICAL VARIATION. ed. 3, p. 10-18, fig. 4 New York, London, 1914.)

KATABOLISM PER UNIT OF SURFACE

Since, however, as appears from the foregoing comparisons, the body surface affords at least as satisfactory a reference unit as body weight, it seems desirable to follow the usual practice and compute the basal katabolism per unit of body surface. Such computations have generally been made by means of the formula proposed by Meeh (*15*)—viz, $S = k\,W^{\frac{2}{3}}$ in which S equals the body surface in square centimeters, W the body weight in grams and k a constant for the same species. Trowbridge, Moulton, and Haigh (*19*) have reported the weights and body surfaces of 35 beef steers and have computed the value of k for different classes of beef cattle. Moulton (*17*) has discussed the data further and has proposed for beef cattle the following modifications of Meeh's formula, which he regards as more accurate, in which W equals the empty weight in kilograms and S the body surface in square meters.

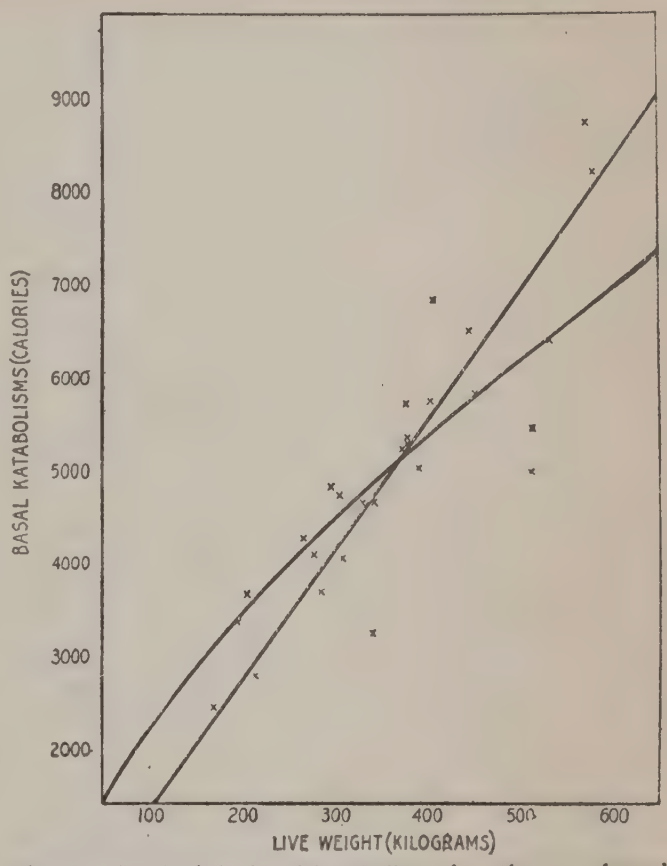

FIG. 2.—Graph of the basal katabolism of cattle per 12 hours' lying and 12 hours' standing.

For cattle in thin or medium condition . . . . . $S = 0.1186\,W^{\frac{5}{8}}$

For fat cattle . . . . . $S = 0.158^{1}\,W^{\frac{5}{8}}$

Applying these formulas to the data of Table I, estimating the empty weight at 89 per cent of the live weight in the unfattened animals and 92 per cent in the full-fed steer C and in steer J, gives the results for the basal katabolism per square meter of surface shown in Table III. For steers C and J the formula for fat cattle has been employed.

The results upon steer J in experiment 217, periods 3 and 4, having been omitted as before, the frequency distribution of the remaining 27 results is shown in figures 4, 5, and 6. While the smoothed graphs show more or less divergence from the probability curve, especially for the results per 24 hours' standing, nevertheless, in view of the rather small number of observations, it would seem that the distribution may be regarded as fairly normal, at least in the two other cases. Assuming this,

[1] Erroneously printed as 0.134 in Moulton (*17*).

the ordinary statistical methods have been applied to the data of Table III, with the results recorded in Table IV. In considering the significance of these results it should be borne in mind that the experiments were upon 10 different animals and that in most instances those upon the same animal were made in different years. How far the factors of individuality and age thus introduced may affect the significance of the statistical calculations is a matter for future investigation. The results are recorded here for what they are worth.

TABLE III.—*Computed 24-hours basal katabolism of cattle per square meter of body surface*

Feeding stuffs.	Experiment No.	Animal No.	Periods compared.	Computed body surface.	Basal katabolism per square meter of body surface.		
					Lying 24 hours.	Standing 12 hours.	Standing 24 hours.
				Square meters.	*Cal.*	*Cal.*	*Cal.*
Timothy hay	174	I	D–A	4.706	1,304	1,472	1,640
Red clover hay	179	I	1–2	5.578	1,074	1,166	1,256
Do	186	I	2a–1a	5.875	1,278	1,412	1,547
Do	186	I	2b–3b	5.825	1,401	1,515	1,631
Timothy hay	190	A	4–3	3.693	802	1,136	1,446
Do	190	B	4–3	2.949	671	1,168	1,182
Do	200	A	4–3	4.690	1,150	1,246	1,338
Do	200	B	4–3	3.925	1,056	1,228	1,407
Do	207	A	4–3	5.437	826	939	1,047
Do	207	B	4–3	4.516	1,157	1,195	1,314
Alfalfa hay	208	D	1–2	2.707	905	933	961
Do	208	E	4–6	3.129	727	916	1,106
Alfalfa hay and mixed grain	208	E	1–3	3.067	960	1,221	1,479
Alfalfa hay	208	C	4–6	3.463	779	1,085	1,398
Alfalfa hay and mixed grain	208	C	2–3	3.340	961	1,308	1,656
Alfalfa hay	209	F	4–6	3.952	909	1,046	1,184
Alfalfa hay and mixed grain	209	F	1–3	3.832	1,048	1,282	1,516
Maize stover	210	D	1–3	4.147	879	1,144	1,405
Mixed hay	211	D	1–5	4.934	1,038	1,338	1,635
Mixed hay and hominy feed	211	D	3–2	5.028	855	1,178	1,498
Mixed hay	211	G	1–5	4.498	1,006	1,293	1,578
Mixed hay and maize meal	211	G	3–2	4.498	1,018	1,211	1,423
Alfalfa hay	212	H	1–5	4.236	862	1,124	1,310
Alfalfa meal	212	H	2–6	4.208	605	788	984
Alfalfa hay	216	J	5–7	4.589	840	1,117	1,382
Alfalfa hay and starch	216	J	1–4	4.458	1,023	1,200	1,381
Alfalfa hay and mixed grain	217	J	2–1	5.452	895	1,020	1,149
Do	217	J	3–4	5.506	1,176	1,370	1,565

TABLE IV.—*Basal katabolism of cattle per square meter of body surface*

	Lying 24 hours.	Standing 12 hours.	Standing 24 hours.
Mean ... Calories	964	1,173	1,365
Probable error of mean ... do	± 24.0	± 21.4	± 25.7
Probable error of single result ... do	±124.8	±110.9	±133.6
Standard deviation ... do	185.1±17.0	164.5±15.1	198.0±18.2
Coefficient of variability	0.1920	0.1462	0.1451

The data of Table III also show a positive correlation between the basal katabolism per square meter of body surface and the live weight, especially for the lying position, as appears from Table V. In other words, the basal katabolism tended to increase more rapidly than the body surface.

TABLE V.—*Coefficients of correlation with live weight*

	Basal katabolism per square meter.
Lying 24 hours	0.5375±0.0923
Standing 12 hours	.3666± .1124
Standing 24 hours	.2405± .1223

MAINTENANCE REQUIREMENT OF CATTLE

In studying the results of feeding experiments it is often desirable to be able to estimate the maintenance requirements of animals. As already pointed out, this is not fixed by the basal katabolism in the narrower sense, but includes also the energy expended in a variety of incidental activities, more or less variable in amount, and corresponding to what we have here called the 24-hour basal katabolism. Our results demonstrate anew the marked influence of standing upon the metabolism of cattle, the mean 24-hour basal katabolism lying, standing 12 hours and standing 24 hours being in the proportion of 100:121:141, the difference largely exceeding the probable errors. For expressing the actual maintenance requirement we have customarily employed the results computed for 12 hours' standing, although this choice is purely arbitrary and any other ratio of standing to lying could be computed from the data of Table IV. If our previous practice be followed, the 24-hour basal katabolism—that is, the maintenance requirement—of an unfattened steer weighing 1,000 pounds (453.6 kgm.), equivalent to 403.7 kgm. empty weight, and standing half the time may be calculated by multiplying the basal katabolism per square

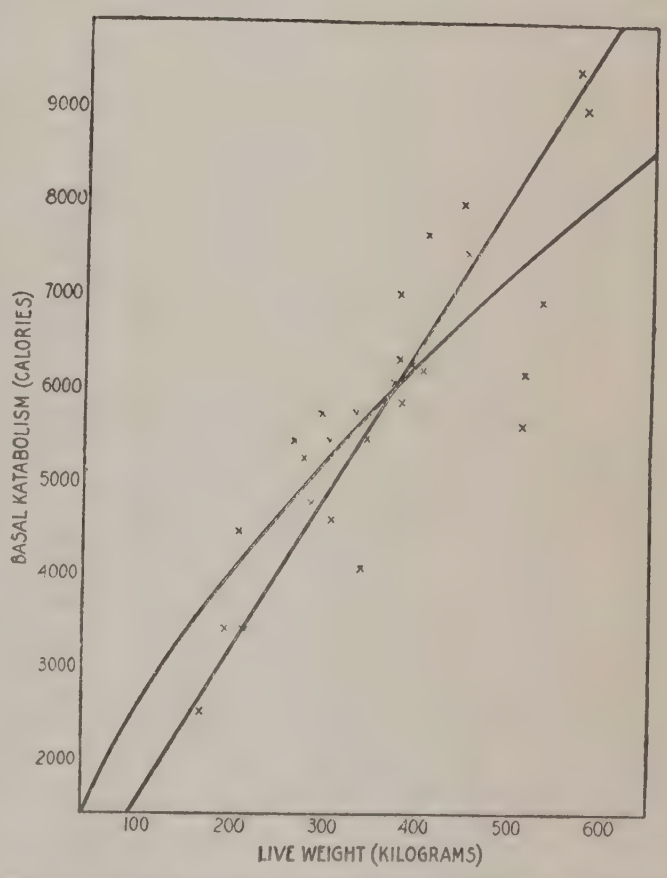

FIG. 3.—Graph of the basal katabolism of cattle per 24 hours' standing.

meter as recorded in Table IV by the body weight as computed by Moulton's formula (p. 47).

$$\text{Maintenance}=1173\pm111\times(0.1186\times403.7^{\frac{2}{3}})=5918\pm560 \text{ Calories.}$$

This result is substantially identical with that computed by the senior author (*2, p. 289*) from 23 of these same experiments in proportion to the two-thirds power of the live weight—viz, 5,906 Calories. No sufficient data seem to be available on which to base a similar computation for fattened animals.

RESULTS ON MAN

Numerous determinations of the basal katabolism of man have been reported, and it appears of some interest to compare their results with our data for cattle. We have not attempted to collate all the recorded experiments on man, but have taken as representative those reported by Benedict, Emmes, Roth, and Smith (*9*) and by Means (*14*), including 98 observation on men and 75 onwomen. These determinations were made in short periods in the post-resorptive condition and in a state of as complete muscular rest as practicable. The majority of them were determinations of the pulmonary respiration made with the Benedict respiration apparatus in periods of 10 to 20 minutes, although some were made with the bed calorimeter and extended over 2 to 3 hours. They therefore show substantially the basal katabolism in the narrower sense mentioned at the beginning of this article.

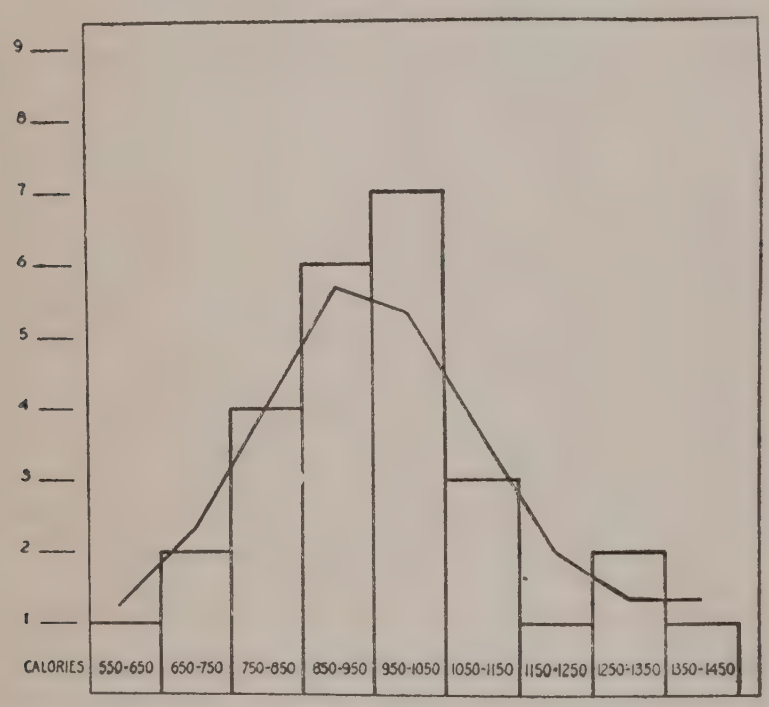

FIG. 4.—Graph of the frequency distribution of the basal katabolism of cattle per square meter of body surface lying 24 hours.

The graphs accompanying Benedict's discussion (*8*) of the results reported in the paper first cited present much the same picture as do our results on cattle as shown in figures 1, 2, and 3, failing to indicate any close relation of basal katabolism to weight or body surface. This conclusion is confirmed by a statistical study of the data, including those reported by Means, which yields the results of Table VI. The correlation coefficients are distinctly lower than those obtained with cattle, but, like them, they fail to show any greater correlation with the body surface as computed by the Meeh formula than with the body weight.

TABLE VI.—*Coefficients of correlation*

Total basal katabolism.	With body weight.	With body surface.
98 men	0.7263±0.0320	0.7747±0.0272
75 women	.7759± .0310	.7447± .0347

For further statistical study the results for the heat production per square meter of surface have been grouped in classes covering a range of 25 Calories in the case of men and 31 Calories in the case of women. The frequency distribution of these classes is shown in figures 7 and 8.

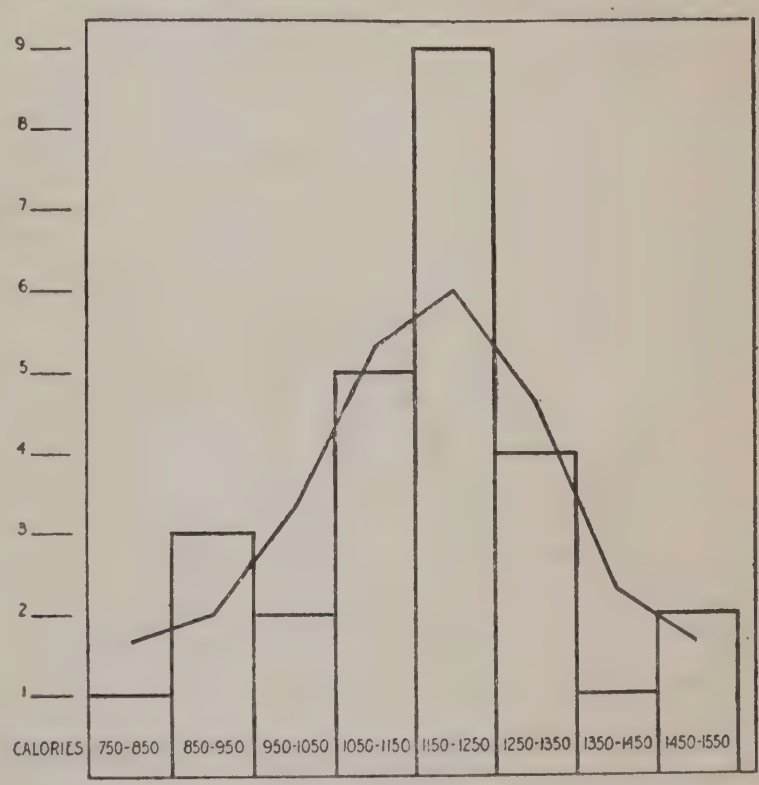

FIG. 5.—Graph of the frequency distribution of the basal katabolism of cattle per square meter of body surface lying 12 hours and standing 12 hours.

The data corresponding to those for cattle recorded in Table IV are contained in Table VII. As would have been expected from the more direct method available in experiments on man, the variability and the probable error are much lower than in the experiments on cattle.

TABLE VII.—*Daily basal katabolism of men and women per square meter of body surface*

	Men.	Women.
Mean ... Calories	830	768
Probable error of mean ... do	±4.3	±4.9
Probable error of single result ... do	±42.3	±42.8
Standard deviation	62.7±3.0	63.5±3.1
Coefficient of variability	.0755	.0827

TABLE VIII.—*Corrected daily basal katabolism of men and women per square meter of body surface*

	Men.	Women.
Mean ... Calories	935	886
Probable error of mean ... do	± 4.8	± 5.8
Probable error of single result ... do	±47.5	±49.4

In all these experiments the body surface was computed by the Meeh formula. D. and E. F. DuBois (*10*) have shown that the use of this formula gives too high results for the body surface of man as compared with direct measurement or with their "linear formula" and have also devised (*11*) a "height-weight chart" for the computation of body surface. Recomputing Benedict's and Mean's results by this latter method, Gephart and E. F. DuBois (*13*) estimate the mean basal katabolism to be 38.97 Calories per square meter per hour for 88 men and 36.9 Calories per square meter per hour for 68 women. Correcting the figures of Table VII in this proportion the basal katabolism per 24 hours and the probable errors are as shown in Table VIII. Considering that these means represent a condition of minimum muscular activity they show a rather striking approximation to that for cattle in the lying position but otherwise free to move.

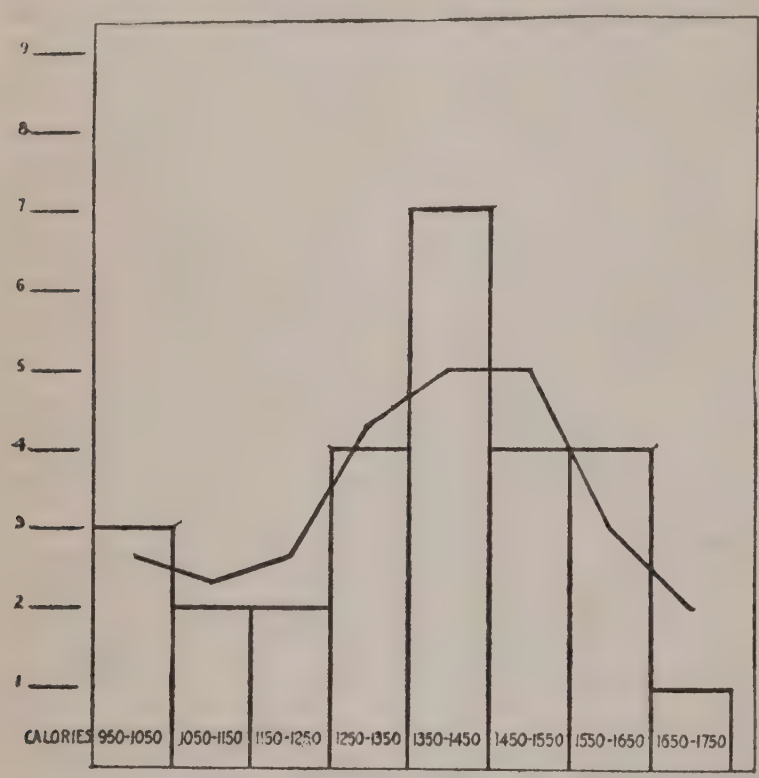

FIG. 6.—Graph of the frequency distribution of the basal katabolism of cattle per square meter of body surface standing 24 hours.

RESULTS ON THE HOG AND THE HORSE

Determinations of the basal katabolism of swine have been reported by Meissl, Strohmer, and Lorenz (*16*) and by Tangl (*18*), while Fingerling, Köhler, and Reinhardt (*12*) have computed it from a comparison of the gains made at two different ages and weights by growing pigs. Their results per square meter of body surface (estimated by Meeh's formula, using for k the value 9.02 found by Hecker for the horse) are recorded in Table IX. In both Tangl's and Fingerling's experiments the animals spent most of the time in the lying position. Meissl makes no statement on this point.

TABLE IX.—*Basal katabolism of swine per 24 hours per square meter of body surface*

Investigators.	State of animal.	Basal katabolism.
		Calories
Meissl et al	Mature animals	1,117
		1,084
Tangl	do	1,063
		1,066
Do	Growing animals	1,139
		1,068
Fingerling et al	do	1,129
		960
Mean		1,078

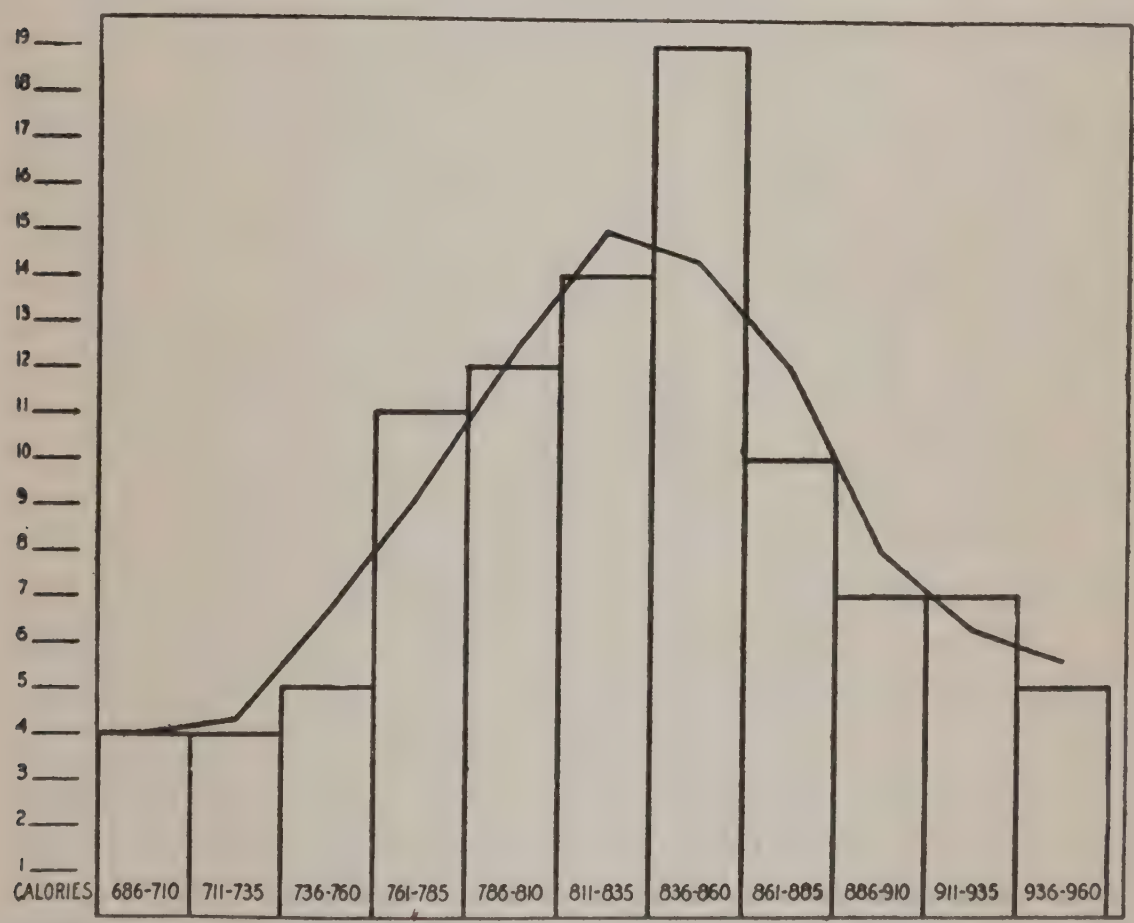

FIG. 7.—Graph of the frequency distribution of the basal katabolism of men per square meter of body surface. Complete muscular rest.

Zuntz and Hagemann (*22*, p. 284) have computed the basal katabolism of the horse from the results of numerous determinations of the respiratory exchange while standing quietly. Their method of computation is in principle the same as that which we have used for cattle, although the experimental methods are entirely different. Their results were as shown in Table X.

TABLE X.—*Computed fasting katabolism of the horse per square meter of body surface per 24 hours*

Period.	Season.	Calories.
a	Winter	807
b	Summer	767
e	Winter	879
f	Summer	936
i	Winter	802
n	Summer	1,085
c	do	976
No. 118c	Winter	1,333
Mean		948

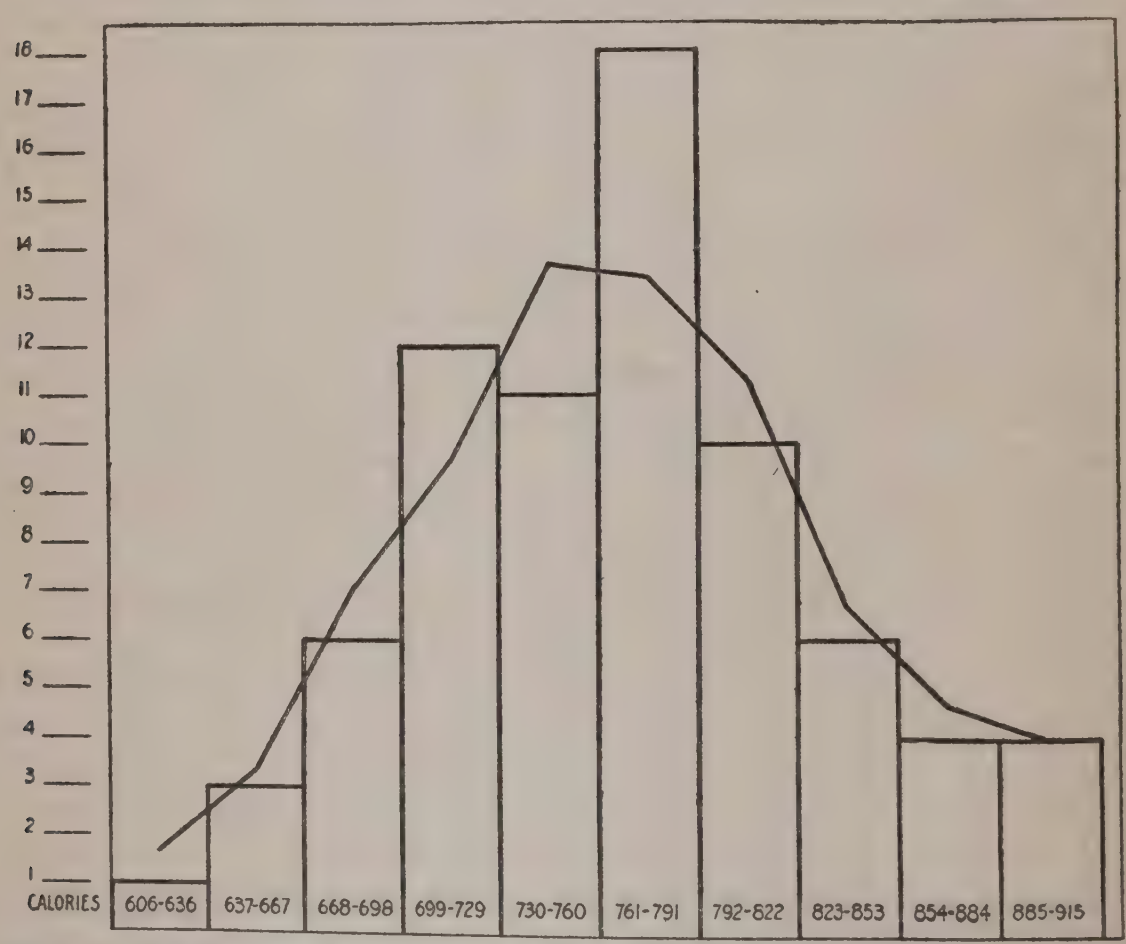

FIG. 8.—Graph of the frequency distribution of the basal katabolism of women per square meter of body surface. Complete muscular rest.

Zuntz and Hagemann regard the lowest of these values, obtained on an exclusive hay ration, as representing the basal katabolism of the horse and regard the higher results as due to a stimulation of the metabolism in periods of light feeding or of low temperature for the sake of heat production—i. e., to a "chemical" regulation of the body temperature.

COMPARISON OF SPECIES

Summarizing the foregoing means from the different species affords the comparisons of Table XI. Considering the nature of the results, they show a rather striking degree of uniformity and tend to confirm the conclusions of Voit (*20*) that the basal katabolism of different species of animals is substantially proportional to their body surface. It may be surmised that the exceptional result with the hog may be due to the imperfect data available for computing the body surface of this species.

TABLE XI.—*Mean daily basal katabolism per square meter of body surface*

Species.	Basal katabolism.
	Calories.
Men (complete muscular rest)	935±5
Women (complete muscular rest)	886±6
Cattle (lying)	964±24
Hogs (lying)	1,078±?
Horse (standing quietly)	948±?

SUMMARY

(1) The results of 27 determinations of the daily basal katabolism of unfattened cattle of different weights and ages are reported.

(2) The basal katabolism, whether computed lying or standing or for an equal proportion of each, was equally well correlated with the estimated body surface and with the live weight.

(3) The basal katabolism per unit of body surface showed considerable variability and a positive correlation with the live weight.

(4) The mean basal katabolism lying, standing 12 hours, and standing 24 hours was in the proportion of 100:121:141.

(5) The mean daily katabolism of a 1,000-pound, unfattened steer standing 12 hours out of the 24 is computed to be 5,918±60 Calories.

(6) Experiments upon man have given results regarding the correlation between basal katabolism and weight or body surface which substantially correspond with those upon cattle.

(7) The mean daily basal katabolism per square meter of body surface appears not to differ greatly in man, cattle, swine, and horses under comparable conditions.

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FURTHER NOTES ON LASPEYRESIA MOLESTA[1]

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INTRODUCTION

Since the publication of a preliminary paper in the Journal of Agricultural Research,[1] investigations on the life history, habits, and control of the oriental peach moth (*Laspeyresia molesta* Busck) have been under way and additional information has been obtained on these points, as well as on its origin, distribution, and food plants.

The fears expressed in the publication cited, namely, that this insect might become a dangerous enemy of deciduous fruits, seem to have been well founded. Owing to the number of generations developing in a single season it is particularly hard to control, and this fact, together with its wide range of food plants, would seem to make it a pest of as great importance as its near relative, the codling moth, *Laspeyresia pomonella* Linnaeus, should it become generally distributed throughout the fruit-growing regions of the country. It is quite probable that it will eventually become widely distributed because of its fruit-feeding habits and its manner of hibernation if measures can not be taken to confine it to its present limits.

ORIGINAL HOME

There is now little doubt that this insect has come to us from Japan, probably in shipments of flowering cherries, or peaches and other fruits, received in the last six or eight years. Where infestations have been found in widely separated localities, shipments of flowering cherries from Japan have, in every instance, been traced to these points. Evidence of this character first led to the belief that the insect came from that country.

In correspondence with Mr. C. Harukawa, of the Ohara Agricultural Institute, Kurashiki, Okayama, Japan, it was learned that a similar insect was doing considerable injury to peaches and pears in that country.

[1] The work on which this paper is based was carried on by the writers under the direction of Dr. A. L. Quaintance, who supervised the operations throughout the season. For assistance in the preparation of the paper the writers express their thanks to Mr. Carl Heinrich, who drew up the description of the larva, furnished the information in regard to characters for separating *Laspeyresia molesta* from the larvæ of similar insects, and approved the drawings of the insect; to Miss Margaret Moles for her careful work in making the drawings; and to Mr. J. H. Paine for the photographs here used.

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Mr. Harukawa stated that the insect had been present there for about 10 years. He very kindly sent specimens of the Japanese insect for comparison and study, and Mr. August Busck, of the Bureau of Entomology, United States Department of Agriculture, determined it as *Laspeyresia molesta*. The evidence seems conclusive that this insect occurred in Japan before its introduction into the United States, but the location of its original home is a matter of conjecture.

FOOD PLANTS

At first it was thought that the only plants attacked were peach and the various cultivated species of Prunus, including cherry, plum, apricot, and several varieties of flowering cherries, but during the past season the insect was reared from quince, pear, apple, and flowering quince. It attacks the quince and apple almost as readily as it does the peach, and the injury caused would undoubtedly be very severe in a large plantation. Of the pome fruits, the quince is the favorite food plant, to judge from the number of insects reared from the fruit. From 10 quinces 93 insects were reared, making an average of more than 9 to each fruit. The late apples also were badly infested. Very little injury was noticed on the twigs of apples, but almost every twig on the quince trees was hollowed at the tip.

DISTRIBUTION IN THE UNITED STATES

From records at hand the insect is present only in the Eastern States. In addition to the locality approximately given as the District of Columbia and adjacent territory it is recorded from northern New Jersey, New York City, Long Island, and Stamford, Conn. Doubtful records come from points near Albany and Buffalo, N. Y., and from southwestern Pennsylvania, but these have not yet been verified. In all localities where the insect has yet been found, with the exception of the vicinity of Washington, D. C., the fruit-growing industry is unimportant and in some places, as in New York City, the only apparent food plants were flowering cherry and flowering quince. If the infestation should extend to regions where fruit is extensively grown and shipped to other parts of the country, the distribution of the insect would almost certainly take place by transportation of the larvæ, either in the fruit or in cocoons on the outside. There is danger also of disseminating the insect by shipping nursery stock bearing hibernating larvæ. Without doubt it was in this way that it first entered the country and reached the localities where it is at present found. It may also spread for short distances from orchard to orchard by flight, as the moth is a strong flier at dusk and in the late afternoon on cloudy days.

CHARACTER AND AMOUNT OF INJURY

The character of injury and amount of damage vary at different seasons of the year, and on different food plants. The damage resulting

from each of the early generations is separated from that occasioned by the next generation by an interval during which no freshly injured twigs can be found. This interval comes in the period between the attainment of full development by the larvæ of one brood and the appearance of the newly hatched larvæ of the next. The interval between the first and second generations and that between the second and third are quite noticeable to one who is making observations in the orchard, but after the third generation the broods of larvæ overlap to such an extent that they can not be thus defined. The injury resulting from each successive generation increases in severity as the season advances, until late summer. In 1917 the number of moths produced by the overwintering larvæ appeared to be small and the amount of injury from the first brood of larvæ was proportionately so, only a few injured twigs showing here and there through the peach orchard. The second-brood injury was much more noticeable and the injury from the third was quite severe. The fourth caused less damage to the twigs than the third, while the fifth brood, appearing late in October, caused almost no injury. In the latter part of the season the insects diminished in numbers on trees without fruit and the overwintering larvæ were few, in comparison to the large number of larvæ of the third generation and the early part of the fourth. It was only on the trees bearing late-ripening varieties of peaches or in the pome fruits that the larvæ of the fourth generation appeared to develop in large numbers.

The injury caused by this insect is of two distinct kinds—namely, injury to the twigs and injury to the fruit. The former is particularly severe on young trees, and occurs mostly before midsummer, while the twigs are yet soft; the latter form does not become severe until after August 1.

TWIG INJURY

The injury to the twigs is first noticed in the spring when the young shoots are about 6 inches long. It is caused by the boring of the larvæ which enter near the tip of the twigs or in the petiole or midrib of the leaves. The injury caused by the newly hatched larvæ may not be noticed for several days after the insect has begun work if the weather is cool and damp, but it appears much sooner if the weather is hot. On peach it usually shows plainest at midday or in the afternoon and is characterized at first by a slight wilting of a single leaf or in some cases the whole tip of the twig and by a very small amount of frass thrown out of the tunnel at the point of entrance. As the insect feeds it increases in size and the tunnel is enlarged accordingly. As the tunneling proceeds the tip of the twig continues to wilt and finally dies. Usually before the twig has completely dried the insect leaves it to find another feeding place or to spin a cocoon if it has fully developed. A larva seldom reaches full development in a single twig unless it be of the thick

type found in some cherries. In slender shoots, such as peach, three, four, or five tips usually will be killed before the larva has matured (Pl. 5, A) Another type of twig injury is found on peach in late summer and fall after the twigs have hardened and stopped growing and after the fruit is gone. This is found usually at the site of previous attack where the gum has exuded and adhered to the bark, sticking fast dead leaves and other débris (Pl. 5, B). In this mass the larva starts work, causing more sap to exude and the twig to swell and in some cases to develop a gall-like formation. The larva mines in the bark and wood of such a twig usually until ready to spin a cocoon. The first six or eight buds below the terminal also may be injured by the late-working larvæ.

The amount of twig injury varies considerably on different food plants, the peach coming first in severity of attack. Young cherries of a number of varieties and the varieties of flowering cherry are very severely injured. Quince probably comes next in the list of injured plants, with plum, apple, pear, nectarine, and apricot following.

FRUIT INJURY

Injury is first noticed in peaches about the time the fruit is the size of chestnuts or slightly larger. In other fruits it has not been noticed so soon. The early injury is caused by larvæ of the second generation, the first-brood larvæ confining themselves almost entirely to the twigs while the fruit is yet small. The second-brood larvæ begin working in the twigs, but when about half grown a few of them turn their attention to the fruit. They bore into the side of the peach (Pl. 6, A, B) and tunnel through the fruit until they are fully developed, emerging sometimes at the point of entrance but most generally through another hole. Such injury usually does not cause the peach to rot or fall to the ground while the fruit is green and hard, but the sap exudes from the wound in the peach, forming a smear of gum on the outside. Frequently the larva, after making a hole in a peach, is apparently "drowned out" by the sap. The sap continues to flow, causing the same gummy appearance of the peach as though the insect had continued to work in the fruit. Most of the third-brood larvæ begin work in the twigs in the same way as the previous broods. A few, however, attack the peach as soon as they are hatched. At this time the early midseason varieties of peaches are ripening and the insect finds it easier to gain entrance to the fruit than when it is green. In a few days nearly all of the insects of this brood desert the twigs for the fruit, and it is at this time that the severe injury to the fruit begins. Varieties of peaches ripening after the 1st of August are all subject to severe injury.

The spot on the fruit most often selected as a point of entry is the area surrounding the stem. When the fruit is beginning to soften the larvæ work beneath the skin at this point and go directly to the seed, leaving in many cases no sign that they have entered. Sometimes

however, a small amount of frass is left at the point of entrance. The small larvæ may not be discovered even after opening the fruit, for at first they work along the grooves in the seeds. Another favorite point of entrance is between two peaches which hang against each other or on the surface of a peach on which a leaf is resting (Pl. 6, A). This seems to give the larvæ a better foothold than they can find on the open surface of the fruit.

The fourth-brood larvæ begin to hatch in time to attack late-ripening Elberta and varieties that ripen still later, Smock being badly damaged. The attack continues until late in the fall after all peaches have been picked.

Larvæ of the fifth generation that hatch early may appear in time to attack the latest-ripening varieties of peaches, but their work is confined mostly to the peach twigs, where they cause little injury, and to the pome fruits, such as quince and apple.

Injury was not noticed on the pome fruits until late in August, and it is thought that before this time they are not heavily attacked. Later, however, the infestation appeared to be serious. From 1½ bushels of medium-sized Ben Davis apples showing signs of injury (Pl. 7, A, B) 354 larvæ of *Laspeyresia molesta* were reared. Fifty per cent or more of the fruit in the orchard from which these apples were taken were injured. In another apple orchard about a half mile distant from the infested peach orchard very little injury was found. Quince was more severely injured than apple (Pl. 6, B). In a row of several trees not a sound fruit could be found, and, as mentioned before, the average number of insects reared from each of 10 quinces, picked at random, was more than 9 per fruit. Even though commercially the injury to pome fruits might not be severe, such food plants are of great importance in that they affect materially the problem of control by furnishing food for the insect in the fall after other fruits have disappeared. The mortality among newly hatched larvæ is probably very great in peach orchards after the twigs have hardened and the fruit is gone, but quinces, apples, or pears furnish an ideal place for the development of the late broods. Such fruits in the vicinity of a peach orchard doubtless form a reservoir from which the infestation spreads the following spring. Cherry and plum ripen too early in the season to be severely injured by this insect. On several cherry and plum trees growing beside an infested peach orchard not one injured fruit was found.

As previously stated, the periods of attack by the first, second, and third generations do not overlap, and there is a period between each generation when no insects can be found working in the twigs or fruit. The larvæ of the third and of the following generations appear over a much longer period of time and before the latest individuals of one generation have developed the larvæ of the following generation are at work. Because of this overlapping of the later broods it was not pos-

sible to tell when one generation had developed fully or when another had begun work, except by rearing the insects through the season under conditions as nearly natural as possible.

Table I shows the dates of the beginning of severe injury to peaches by each brood of larvæ, the percentages of fruit injured as shown by counts in definite periods throughout the summer, and a few varieties of peaches ripening in each period.

TABLE I.—*Dates of injury to peaches by respective broods of larvæ of the oriental peach moth, Arlington Farm, Va., 1917*

Date of beginning of severe injury.	Percentage of peaches injured in period from—	Common varieties of peaches ripening in periods named in column 2.
By first generation, May 31.	May 31 to June 30....0.	Greensboro.
By second generation, July 6.	July 1 to July 15...2.5.	Greensboro. Waddell.
	July 16 to July 31....3.	Carman. Hiley.
By third generation, July 28.	Aug. 1 to Aug. 15...18.	Champion. Early Crawford. Bell. Old Mixon Free. Reeves.
	Aug. 16 to Aug. 31..28.2.	Elberta. Late Crawford. Chairs.
By fourth generation, Aug. 30.	Sept. 1 to Sept. 16...53.	Smock. Stump.
By fifth generation, Oct. 7.	Sept. 17 to Oct. 20, no count made.	Salway. Bilyeu.
	All fruit harvested.....	

INSECTS LIKELY TO BE CONFUSED WITH THE ORIENTAL PEACH MOTH

There are several insects which may be confused with *Laspeyresia molesta* in the larva stage, either because of a close resemblance or because of a similarity in the injuries which they cause. The more common of these are the codling moth, *Laspeyresia pomonella* Linnaeus; the lesser apple worm, *L. prunivora* Walsh; the peach twig borer, *Anarsia lineatella* Zeller; and *Laspeyresia pyricolana* Murtfeldt.

L. pomonella is likely to be mistaken for *L. molesta* in the fruit of the apple, pear, and quince, but close examination will show several points of difference in the mature larvæ. The following characters serve to separate the larvæ of the two species: On *L. pomonella* (Pl. 8, G) the anal fork is absent; a scobinated pad is present extending across the anal proleg just in front of the crochets; the crochets on abdominal prolegs are 23 to 38 in number. On *L. molesta* (Pl. 8, F) the anal fork is present, situated just below the anal plate and above and behind the anal prolegs; the scobinated pad is absent; the crochets are 31 to 46 in number. The full-grown larva of *L. molesta* is smaller than that of *L. pomonella*.

L. prunivora works in apple, causing injury to fruit almost identical with that caused by *L. molesta* (Pl. 7, A); it does not injure twigs. Specimens of larvæ were not available for comparison. Superficially they appear the same as *L. molesta*.

Anarsia lineatella attacks peach twigs, causing injury identical with the spring injury of *L. molesta* (Pl. 5, A). The larva is most readily separated from *L. molesta* by the setal plan of the ninth abdominal segment, which should be compared with that shown in Plate 8, D. In *Anarsia lineatella* seta I is not approximate to seta III, being farther from or at least as far from seta III as from seta II; the frons extends almost to the incision of the dorsal hind margin; the longitudinal ridge is extremely short; setæ P_1 and P_2 and puncture P_b lie in a line; P_2 is well behind the level of Adf_2.

Laspeyresia pyricolana attacks the twigs of apple, boring out the center and killing the tip. The injury resembles that of *L. molesta* on apple twigs. No specimens were available for comparison.

LIFE HISTORY AND HABITS

The following data on the life history of *Laspeyresia molesta* are based on material collected chiefly at the United States Department of Agriculture experimental farm near Rosslyn, Va., and reared in the insectary at the Bureau of Entomology, Washington, D. C., during the season of 1917. The insectary provides practically outdoor conditions where insects may develop normally.

SPRING EMERGENCE AND OVIPOSITION

In mid-March hibernating larvæ pupate and about mid-April, or when peaches are in full bloom, the first adults emerge, their emergence continuing through the first three weeks of May. The time elapsing between emergence and the beginning of oviposition, called the preoviposition period, ranges from 2 to 12 days and averages 5 days. In 1917 the first eggs were found in a peach orchard on May 3. Normally the eggs are deposited singly on the under side of the leaves, and in the orchard they were not found in any other place. In glass rearing jars an occasional egg was deposited on the upper surface of the leaf, and in one case four eggs were found on the bark of a peach seedling confined in a rearing cage. The moths oviposit much more freely on the smooth glass surface of the battery jar and lantern globe rearing cages than on peach foliage placed in the jar; hence eggs used for study were those deposited on the glass of the rearing cages.

The deposition of eggs began May 2 and continued until late in the fall. The last egg observed was found October 8. At this time development had proceeded far enough to show the eye-spots in the embryo.

THE EGG

The egg (Pl. 9, B), is scalelike, oval, slightly convex, flattened toward the edge; color grayish white, somewhat iridescent; average measurement 0.59 by 0.72 mm. Central surface finely granulate with reticulating ridges extending from the edge toward, but not to, the center.

The average incubation periods in 1917 for eggs of the first three generations, respectively, were 7.5, 4, and 3.1 days. For eggs of the fourth and fifth generations, collectively, the incubation period was 8.3 days.

The progress of development in the egg can be readily observed through its thin shell. In midsummer the progress is so rapid that the embryonic outline is easily discernible 12 hours after deposition. The darkening of the head is first evidenced by the appearance of eyespots. A large majority of the eggs hatch in the late afternoon, during periods when the temperature is high enough to insure steady development. When ready to be hatched the young larva makes rather vigorous movements of its head and mandibles against the eggshell, which finally is slit open and the larva walks out. In one instance when the hatching of an egg was closely observed, 57.5 minutes elapsed from the time of the first movement of the mandibles of the larva until it had entirely quitted the eggshell.

THE LARVA

The larva (Pl. 9, A) is cylindrical; without secondary hair; color varying from white to deep pink, usually more strongly suffused with pink on dorsal side. Legs and prolegs normal. Crochets (31 to 46) uniordinal, in a complete circle. Anal fork developed, yellow to black in color, three to six pointed, prominent. Setal areas broadly chitinized, grayish brown. Thoracic shield light yellow edged with yellowish brown, narrowly divided, moderately broad. Spiracles dark brown or black, small, circular, slightly produced; spiracle on prothorax and that on abdominal segment 8 very little larger than those on abdominal segments 1 to 7. Entire body, except chitinized areas, evenly and finely scobinate; what appears to be a coarse pubescence under low magnification proves, under high magnification, to be a mass of short aculei.

Body setæ (Pl. 8, D) yellow shading to deep brown, moderately long. Prothorax with Ia and Ib on, and Ic behind the anterior margin of the shield; IIa and puncture y caudad of Ia; IIb directly laterad of IIa; puncture x dorsad of and approximate to Ib, lower than the level of IIb; Ib, Ic, and IIc equidistant; prespiracular shield oval, situated ventro-cephalad of the spiracle, bearing three setæ; group VI bisetose. Mesothorax and metathorax with VI unisetose. Abdominal segments 1 to 7 with II longer than and ventro-caudad of I; III over the spiracle; IIIa approximate to III, dorsocephalad of the spiracle; IV and V on the same chitinization, under the spiracle, approximate. Abdominal segment 8 with II only slightly below the level of I; III and IIIa cephalad of the spiracle. Abdominal segment 9 with all setæ in a line, I and III closely approximate; V, IV, and VI on the same chitinization, approximate; VII unisetose.

Head light brown, with darker brown mottling; hind margin, ocellar area, and tips of trophi black.

Head capsule (Pl. 8, A, B, C) nearly spherical, slightly flattened, broadly oval in outline viewed from above, a little wider than long; greatest width well behind the

middle; incision of dorsal hind margin about one-fourth the width of the head; distance between dorsal extremities of hind margin less than one-half the width of the head. Frons (Fr) only slightly longer than wide, reaching to middle of head; adfrontal ridges (AdfR) sinuate; longitudinal ridge half the length of the frons; adfrontal suture (AdfS) reaching to dorsal incision of hind margin. Projection of dorsal margin over ventral slightly less than one-third the diameter of the head.

Ocelli six, in the normal tortricid arrangement; III, IV, and V in a straight line; I larger than the others.

Epistoma with the normal setæ (E_1, E_2).

Frontal punctures (F^a) lying rather closely together, anterior to the setæ (F_1); distance between punctures less than from puncture (F^a) to seta (F_1); adfrontal seta (Adf_1) nearer to F_1 than to Adf_2; adfrontal puncture (Adf^a) approximate to Adf_2.

Epicranium with the normal number of primary setæ and six punctures, and with three small ultraposterior setæ and one ultraposterior puncture. Anterior and lateral setæ (A_1, A_2, A_3, and L_1) in a line, with distances between A_1 and A_2, A_2 and A_3, and A_3 and L_1 about equal; puncture (A^a) postero-dorsad of A_2; A_1, A_2, and A_3 on a level respectively with F^a, F_1 and Adf_1. Posterior setæ (P_1 and P_2) and punctures (P^a and P^b) at middle of head; P_1 on a level with adfrontal puncture (Adf^a); P_2 and puncture (P^b) on a level with beginning of longitudinal ridge (LR); P_2, P_1, and adfrontal seta (Adf_1) in a line; puncture (P^a) approximate to and equidistant from A_3 and L_1. Lateral seta (L_1) on a line with P_1 and adfrontal puncture (Adf^a); lateral puncture (L^a) directly posterior to the seta. Ocellar setæ (O_1, O_2, O_3) well separated. O_1 closely approximate to and equidistant from ocelli II and III, within the area bounded by the ocelli; O_2 closely approximate to and postero-ventrad of ocellus I; O_3 postero-ventrad of and remote from O_2, slightly below the level of ocellus VI; puncture O^a absent. Subocellar setæ (So_1, So_2, So_3) triangularly placed. So_2 and So_3 closer together than So_2 and So_1; puncture (So^a) lying midway between So_2 and So_3. Genal seta (G_1) and puncture (G^a) both present; puncture anterior to the seta.

Length of full-grown larva 11 to 13 mm.

When the young larva hatches it immediately starts on its search for a favorable feeding place. In one instance 20 minutes were required after hatching for a larva to explore three peach leaves and to make its way to the tender growth at the terminal, where it bored into the interior of the peach shoot. The larvæ do not feed as they enter, but withdraw their heads from the burrow and cast aside the fragments of tissue until the more succulent interior of the twig is reached. If the young larvæ fail to locate favorable feeding places in a short time they undoubtedly die, for in the rearing jars they die within 12 hours after hatching.

The length of time required for larvæ to develop fully varies considerably, the feeding period being from 8 to 16 days in length throughout the entire season and the average for 59 larvæ being 11.2 days. When the larva has fully developed it leaves the twig or fruit where it has been working and starts in search of a favorable place for spinning its cocoon. The spring and midsummer cocoons are formed mostly in the axils between twigs or on the fruit at the point where it is attached to the stem. The latter place is the one most often chosen. Occasionally a larva will spin on the open surface of the peach, but usually it selects a more sheltered spot. The cocoon is made of fine silken strands, the

exterior mixed with fragments of frass or of bark, or if it is on the surface of the peach the fuzz or pubescence from the skin of the peach will be incorporated in the cocoon. Occasionally in a dry fruit, such as quince, the insect will spin its cocoon inside of the fruit. The time from the spinning of the cocoon to pupation is from 2 to 9 days and the average 3 days.

THE PUPA

The pupa (Pl. 9, C, D, E) is yellow-brown in color; without pubescence; average measurements (3 specimens) 6.26 mm. long by 1.8 mm. wide. Frontoclypeal suture indistinct; eyes and glazed eyes discernible; mandibles and clypeus distinctly indicated; 2 pairs of clypeal setæ, inner pair slightly longer than outer; clypeo-labral suture not visible; labial palpi slightly more than half the length of the maxillæ; maxillary palpi extending to the proximo-lateral angles of the maxillæ; maxillæ reaching one-third of the way to the tips of the wings. Metathoracic legs and tips of hind wings reaching just beyond the cephalic edge of the fourth abdominal segment; antennæ extending about two-thirds of the wing length, reaching beyond second coxæ. A double row of dorsal spines on abdominal segments 2 to 7; abdominal segment 2 with spines of cephalic row uneven in size and arrangement, the row extending usually less than half-way across the segment, the caudal row well developed and extending almost across; segments 3 to 7 with spines of cephalic row about twice as large and half as numerous as those of the caudal row; segments 8 to 10 with one row of spines, the spines gradually increasing in size from segment 8 to segment 10.

No cremaster. Two hooked setæ on either side of the anal rise, with a third hooked seta latero-caudad; a fourth pair of hooked setæ, dorso-caudad of the third pair, is on the caudal margin of the abdomen. Spiracles circular and produced. Anal and genital openings slitlike, the latter single in both sexes.

The pupa period covers from 5 to 12 days, averaging 7.8 days. When the moth is ready to emerge the pupa pushes itself from its cocoon by means of rows of dorsal spines on the abdomen. When it protrudes from the cocoon far enough to permit emergence of the moth the pupa fastens itself to the inside of the cocoon by means of hooked spines arranged upon the caudal margin. The pupal case then splits in the cephalic and thoracic regions, permitting the moth to emerge.

THE ADULT

The head of the adult[1] (Pl. 10, A, B, C) is a dark, smoky fuscous; face a shade darker, nearly black; labial palpi a shade lighter fuscous; antennæ simple, rather stout, half as long as the forewings, dark fuscous with thin, indistinct, whitish annulations. Thorax blackish fuscous; patagia fainly irrorated with white, each scale being slightly white-tipped. Forewings normal in form; termen with slight sinuation below apex; dark fuscous, obscurely irrorated by white-tipped scales; costal edge blackish, strigulated with obscure, geminate, white dashes, four very faint pairs on basal half and three more distinct on outer half besides two single white dashes before apex; from the black costal intervals run very obscure, wavy, dark lines across the wing, all with a strong outwardly directed wave on the middle of the wing; on the middle of the dorsal edge the spaces between three of these lines are more strongly irrorated with white than is the rest of the wing, so as to constitute two faint and poorly defined,

[1] Description by Mr. August Busck. (QUAINTANCE, A. L., and WOOD, W. B. LASPEYRESIA MOLESTA, AN IMPORTANT NEW INSECT ENEMY OF THE PEACH. *In* Jour. Agr. Research, v. 7, no. 8, p. 373-374. 1916.)

white dorsal streaks. All these markings are only discernible in perfect specimens and under a lens; ocellus strongly irrorated with white, edged by two broad, perpendicular, faint bluish metallic lines and containing several small deep black, irregular dashes, of which the fourth from tornus is the longest and placed farther outward, so as to break the outer metallic edge of ocellus; the line of black dashes as well as the adjoining bluish metallic lines are continued faintly above the ocellus in a curve to the last geminate costal spots; there is an indistinct, black apical spot and two or three small black dots below it; a thin but distinct, deep black, terminal line before the cilia; cilia dark bronzy fuscous. Hind wings dark brown with costal edge broadly white; cilia whitish; underside of wings lighter fuscous with strong iridescent sheen; abdomen dark fuscous with silvery white underside; legs dark fuscous with inner sides silvery; tarsi blackish with narrow, yellowish white annulations.

Alar expanse: 10 to 15 mm.

United States National Museum type 20664.

Adults emerged in 1917 from April 16 until October 30, though only a few straggling individuals emerged after October 5.

The preoviposition period for the entire season varied from 2 to 12 days, averaging 4.7 days, and there is some evidence that oviposition occurred in a few instances the day following emergence.

In the rearing cages the moths are quiet during the day, but become active during late afternoon and early dusk. Oviposition began in a few cases between 3 and 4 o'clock in the afternoon, and it usually continues throughout the dusk of evening.

In order to obtain eggs it was necessary to confine more than one pair of moths in each rearing cage. In one instance eggs were obtained in a jar containing one female and three male moths, but most satisfactory results were obtained by confining about 20 moths with a representation of both sexes in each jar.

In no case were eggs produced by isolated pairs. The recorded number of eggs deposited in rearing jars varied from 1 to 391. The single egg was produced in a jar containing 3 female moths and 1 male. A jar containing 12 female and 8 male moths produced the 391 eggs.

Adults are seen infrequently in the orchard during the day, but from late afternoon to late dusk they fly about the upper parts of the peach trees and in sheltered places between the trees. They are most active during early dusk. In the first part of August they appeared in such numbers that they were easily noticed, and by early September they were observed flying in large numbers. Their flight is rapid, erratic, and irregular, though occasionally they dart away in a definite direction. Moths thus seen actively flying were nearly all males. Of eight captured on the evening of August 20, four were females. One stroke of a collecting net captured them from a twig where two moths were seen to alight. Only one other female moth was captured in the field, though a large number of males were taken during August and September. The females in rearing cages fly as vigorously as the males, and there is little doubt that distribution of the insect throughout orchards and from one orchard to another takes place rapidly by means of flight.

HIBERNATION

The insect hibernates in the larva stage in cocoons spun in the autumn after the larvæ have fully developed. In the peach orchard a large percentage of the overwintering insects spin their cocoons in small cracks in the bark, under bark flakes, and in curled ends of bark strips on the trunk and large branches of the trees. The range of places for spinning is shown in the following list of locations in which cocoons were observed in April, 1917, at the United States Department of Agriculture experimental farm near Rosslyn, Va.: (1) Under edges of bark scales; (2) in axils of fruit spurs; (3) in the curled ends of scales of bark; (4) beneath scales at axils of secondary branches; (5) between mummied peaches on the trees and on the ground; (6) between peaches and the spur bearing them; (7) in old bark wounds; (8) in the frass at enlarged ends of twigs fed upon last season by the larvæ; (9) in the wrinkles of mummied peaches on the ground; (10) on the smooth bark of the twigs; (11) in burrows made by barkbeetles; (12) in holes formerly filled with pith at end of stub made by pruning; (13) in the hollows of stubble.

PARASITES[1]

Eight species of hymenopterous parasites have been reared. Six of them are primary and two are secondary parasites. One dipterous parasite, *Hypostena variabilis* Coquillett, was reared from larvæ collected in the orchard. It pupated within the partially constructed cocoon of the host. The host was probably attacked while the larva was seeking a cocooning place.

Of the six primary hymenopterous parasites, *Macrocentrus* sp. was most abundant. *Macrocentrus* sp. (Q. 7897) attacks and develops within feeding larvæ of *Laspeyresia molesta*, spinning its cocoon within the cocoon of the host. The latter may be thin and unfinished, due to the weakened condition of the larva. This species is also a parasite of the codling moth, *L. pomonella*. *Phaeogenes* sp. (Q. 7204) was second in abundance. Phaeogenes emerges from the pupa of the host and probably attacks the insects in the prepupa or pupa stage. Several specimens of *Ascogaster carpocapsae* Viereck were reared. According to Mr. Cushman, *A. carpocapsae* oviposits in the egg of the host, kills the insect as a larva after it has spun its cocoon, and spins its own cocoon within that of the host. One specimen each of *Spilocryptus* sp. (Q. 6833), *Mesostenus* sp. (Q. 1345), and *Glypta vulgaris* Cresson were reared. Spilocryptus attacks the host after the larva has spun its cocoon, and the adult parasite emerges from the pupa of the host. Glypta and Mesostenus attack the feeding larva and kill the host in the prepupa stage. Each one spins its cocoon within the cocoon of the host.

[1] Through the assistance of Mr. R. A. Cushman, of the Bureau of Entomology, United States Department of Agriculture, the writers are enabled to give the breeding habits of the parasites and the relation of the parasites to the host.

Of the two secondary parasites, *Dibrachys boucheanus* (Ratzeburg) pupated within and was reared from cocoons of *Macrocentrus* sp., already mentioned as a parasite of *L. molesta*. Of three specimens of *Cerambycobius* sp. (Q. 5195), two were found in cocoons of *Macrocentrus* sp.

OTHER NATURAL ENEMIES

A small spider was observed to kill a partly grown larva of *L. molesta*. The larva was evidently migrating from one feeding place to another when captured. On two occasions larvæ of lacewing flies were seen with the larvæ of *L. molesta* in their mandibles. In a few instances cocoons were found torn open and contents removed, evidently by woodpeckers.

CONTROL MEASURES

Because of its habit of feeding inside of the twigs and fruit, no success was obtained in controlling the insect on peaches by the use of poisoned sprays. Arsenate of lead, though applied to the fruit, foliage, and twigs just before the eggs were due to hatch, did not prevent the larvæ from entering the twigs and fruit and gave no degree of control. Other applications in addition to this one, made at such times as it was thought the insect would be most vulnerable to attack, gave no better results in control. A 40 per cent nicotine sulphate solution, diluted to 1 part in 400 parts of water and applied in the same way and at the same time as the treatments with arsenate of lead, did not control the insect; although counts made early in the season of the number of infested twigs on the sprayed and unsprayed plats seemed to indicate slight benefit from the treatment. A combination spray of lead arsenate and nicotine sulphate likewise gave negative results.

Banding the trees with burlap resulted in the capture of a few larvæ, but most of the insects, after leaving the twigs and fruit, spin their cocoons around the fruit spurs, on the peaches, and in the axils of the twigs, thus making this operation a failure.

Clipping the infested twigs from the trees and destroying them, and destroying infested fruit, gave partial control, but was too laborious to be practical.

Tests were made of the killing power of miscible oils and nicotine sulphate when applied to the cocoons containing overwintering larvæ, and when applied directly to the insects by immersing them in the liquid. A number of larvæ in cocoons were immersed for 17 hours in a miscible oil diluted in water at the rate of 1 part in 10. Several hours after removal from the solution one-third of the larvæ appeared to be uninjured and were spinning new cocoons. The balance were almost inactive but none was dead and none died within a week. A similar test was made using 40 per cent nicotine sulphate at a dilution of 1 to 233 combined with the oil solution used above. The larvæ after removal

were all alive but were inactive and none died within 48 hours. About one-third of the insects were alive a month later. Other tests were made for shorter periods of time without satisfactory results.

Fumigation tests with hydrocyanic-acid gas[1] were made on overwintering larvæ in cocoons. The heaviest dosage used with natural atmospheric pressure was 1 ounce of sodium cyanid to 100 cubic feet of space for a period of one hour. With such treatment the larvæ were not killed. Other tests were made in which were used larvæ taken from the orchard in December in enlarged gummy twigs. The larvæ were incased in a hard mass of dried gum and leaves. They were fumigated in a 25-inch vacuum with a dosage of 1 ounce of sodium cyanid to 100 cubic feet of space for one hour, and also with a dosage of double this amount for two hours. Neither treatment killed all of the larvæ.

From the results obtained in the dipping and fumigation tests noted above it would appear to be impossible to free infested nursery stock from this insect by such means.

Parasitism appears to play an important part in controlling the pest, and the attack in the latter part of the season undoubtedly lessens to an appreciable extent the number of moths emerging in the following spring, but sufficient data on the percentage of parasitism occurring have not yet been collected to warrant a definite statement in this regard.

[1] Tests made by Mr. E. R. Sasscer, of the Federal Horticultural Board.

PLATE 5

Laspeyresia molesta.

A.—Peach twig showing summer injury.

B.—Peach twig with mass of gum, leaves, and frass; a type of injury found in fall and winter.

A
B

A
B

PLATE 6

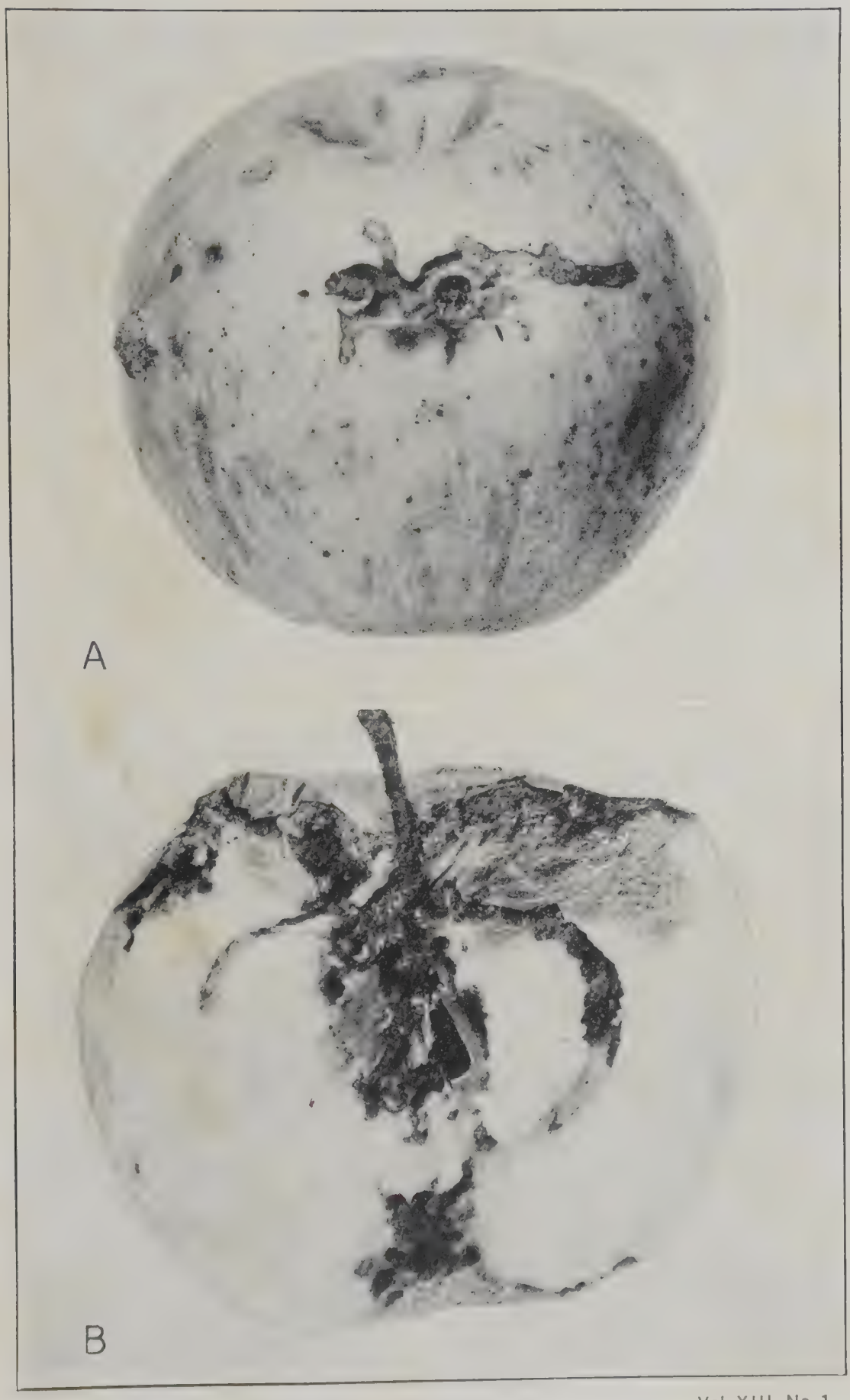
Laspeyresia molesta:

A.—A green peach attacked by the caterpillar, illustrating a common type of injury.
B.—A quince severely injured.

PLATE 7

Laspeyresia molesta:

A.—Typical injury by larva on apple, resembling that caused by *Laspeyresia prunivora.*
B.—Injury to the interior of the fruit.


A
B

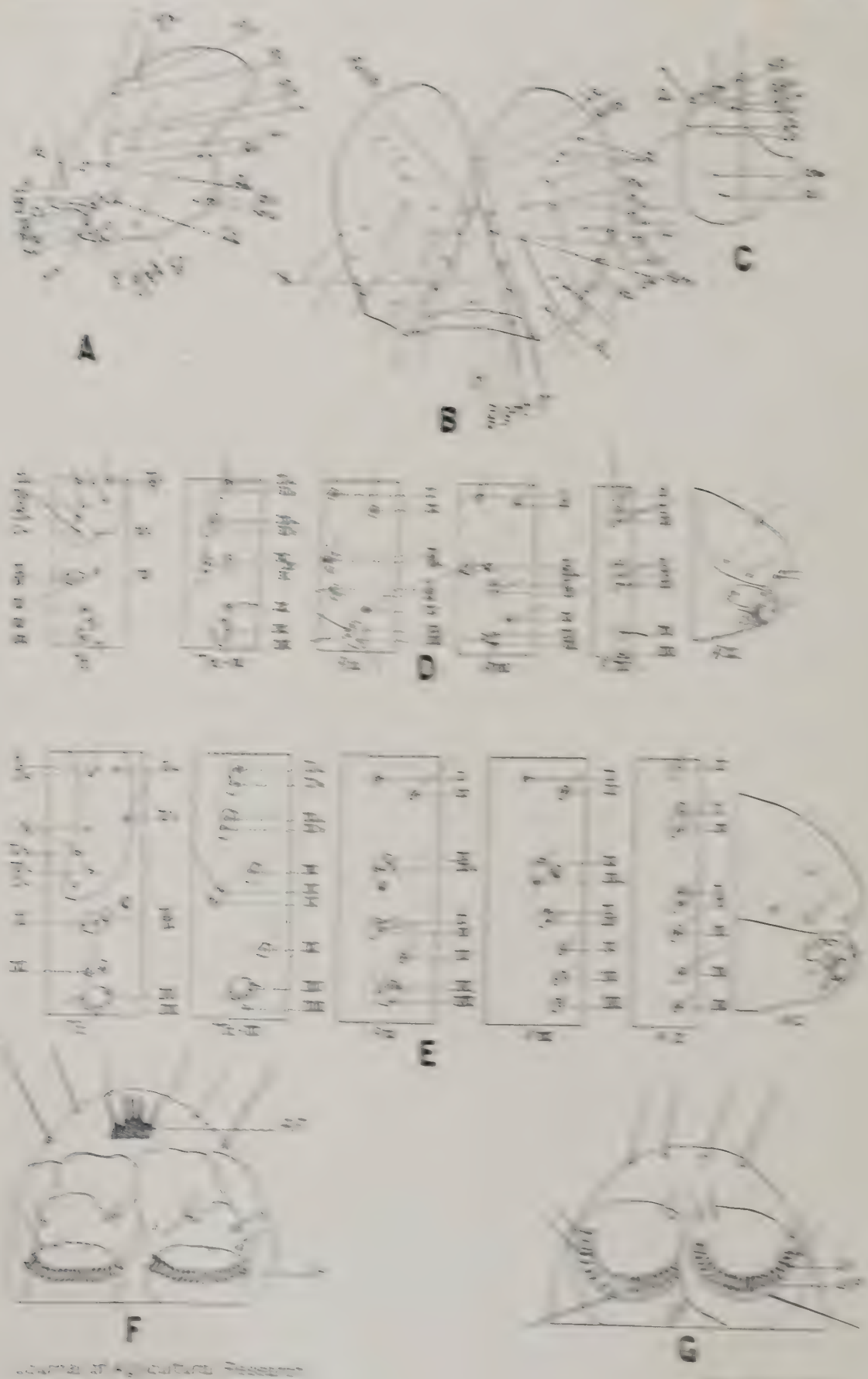

A
B
C
D
E
F
G

PLATE 8

A.—*Laspeyresia molesta:* Head capsule of larva from side.

B.—*Laspeyresia molesta:* Head capsule of larva from front.

C.—*Laspeyresia molesta:* Head capsule of larva from beneath. A_1, Anterior seta 1; A_2, anterior seta 2; A_3, anterior seta 3; A^a, anterior puncture; Adf_1, adfrontal seta 1; Adf_2, adfrontal seta 2; Adf^a, adfrontal puncture; *AdfR*, adfrontal ridge; *AdfS*, adfrontal suture; E_1, epistomal seta 1; E_2, epistomal seta 2; F_1, frontal seta; F^a, frontal puncture; *Fr*, frons; G_1, genal seta 1; G^a, genal puncture; L_1, lateral seta 1; L^a, lateral puncture; O_1, ocellar seta 1, O_2, ocellar seta 2; O_3, ocellar seta 3; P_1, posterior seta 1; P_2, posterior seta 2; P^a, posterior puncture *a*; P^b, posterior puncture *b*; So_1, subocellar seta 1; So_2, subocellar seta 2; So_3, subocellar seta 3; So^a, subocellar puncture; *X*, ultraposterior setæ and punctures; *I*, ocellus 1; *II*, ocellus 2; *III*, ocellus 3; *IV*, ocellus 4; *V*, ocellus 5; *VI*, ocellus 6.

D.—*Laspeyresia molesta:* Diagram showing arrangement of body setæ on segments. T_I, first thoracic segment; T_{II+III}, second and third thoracic segment; A_{III}, third abdominal segment; A_{VIII}, eighth abdominal segments; A_{IX}, ninth abdominal segment; A_X, tenth abdominal segment.

E.—*Laspeyresia pomonella:* Chart showing arrangement of body setæ on segments. The numbering of segments is the same as in D.

F.—*Laspeyresia molesta:* Ventral view of anal prolegs and caudal end of abdomen. *AF*, anal fork; *Cr*, crochets.

G.—*Laspeyresia pomonella:* Ventral view of anal prolegs and caudal end of abdomen. *Cr*, crochets; *SP*, scobinated pad.

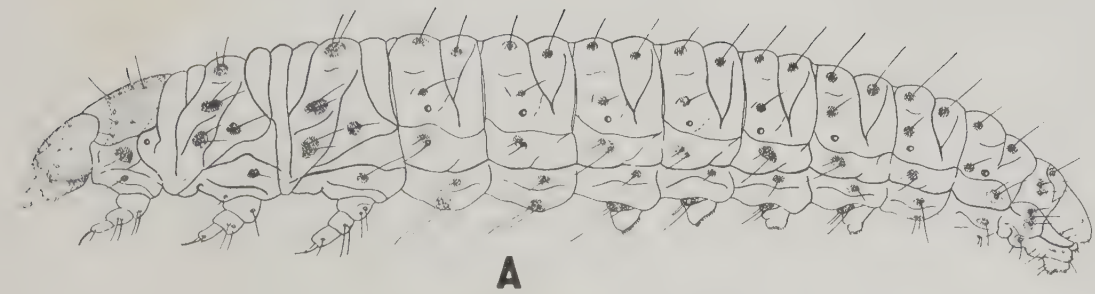
PLATE 9

Laspeyresia molesta:

A.—Larva.

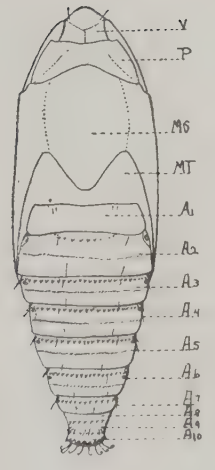
B.—Egg.

C, D, and E.—Pupa, dorsal, lateral, and ventral views. A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8, A_9, A_{10}, abdominal segments 1 to 10; *AO*, anal opening; *Cl*, clypeus; *Cx2*, mesothoracic coxa; F_1, prothoracic femur; *GE*, glazed eye; *GO*, genital opening; L_1, prothoracic leg; L_2, mesothoracic leg; L_3, metathoracic leg; *LP*, labial palpus; *Md*, mandible; *MP*, maxillary palpus; *MS*, mesothorax; *MT*, metathorax; *Mx*, maxilla; *P*, prothorax; *V*, vertex; W_1, mesothoracic wing; W_2, metathoracic wing.



A

B


V
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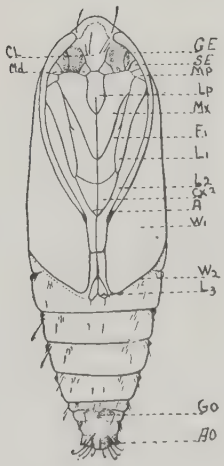
C

D


Cl
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Mx
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L1
L2
Q2
A
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L3
GO
AO

E

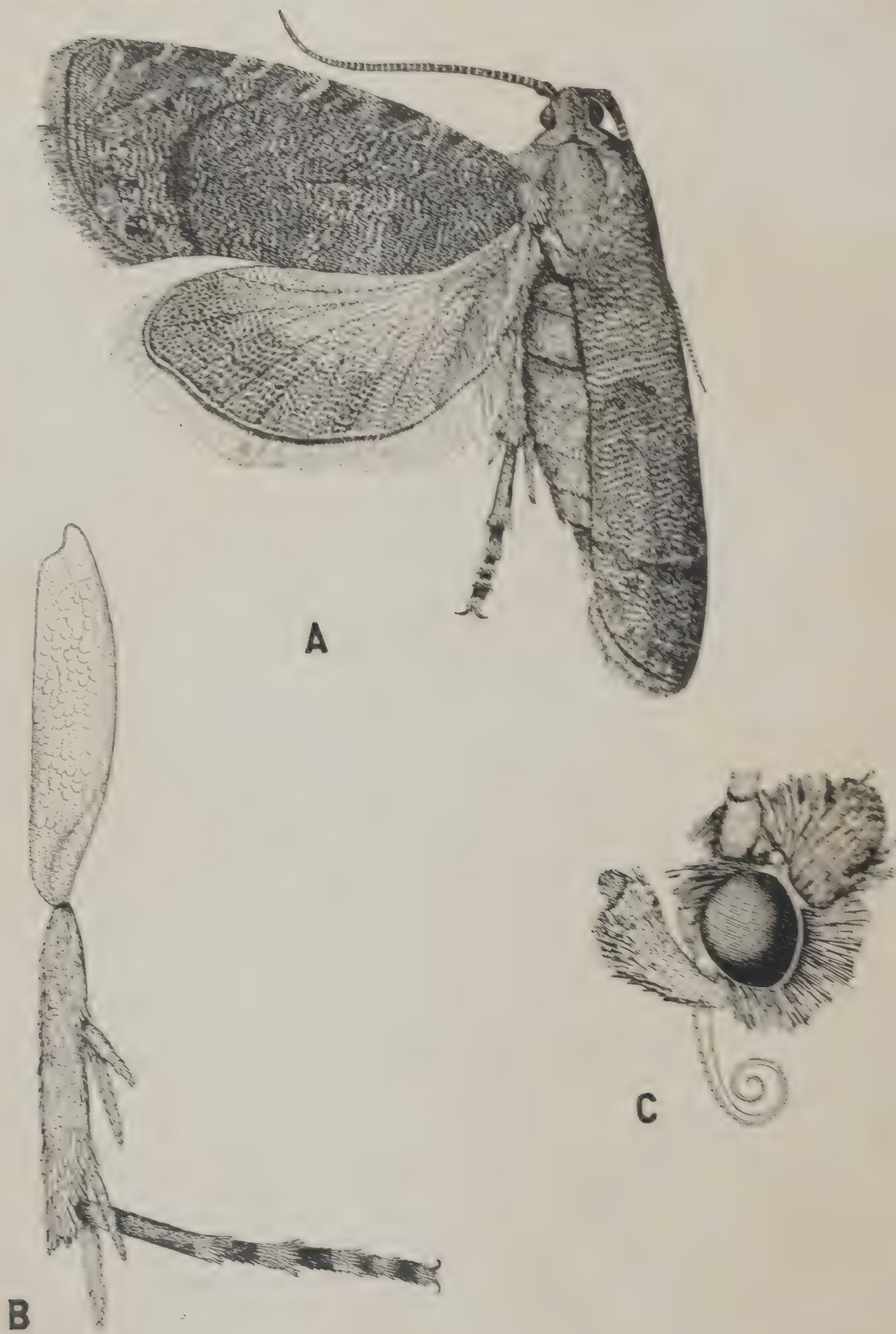
A
B
C

PLATE 10

Laspeyresia molesta:

A.—Adult.
B.—Metathoracic leg.
C.—Head and mouth parts.

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SOIL FUNGI IN RELATION TO DISEASES OF THE IRISH POTATO IN SOUTHERN IDAHO

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INTRODUCTION

In a former paper it was shown that planting disease-free seed potatoes (*Solanum tuberosum*) on lands never before in cultivation could not be considered a guarantee of a disease-free product (*10*).[1] Research studies with the powdery-dryrot organism (*Fusarium trichothecioides* Wollenw.) (*9*) and with *Fusarium radicicola* Wollenw., the cause of a blackrot (*11*) of the Irish potato tuber, indicated that these organisms might be well distributed in the desert soils. From the results of the 1915 plantings it was assumed that the infection which was observed in the harvested crop must have proceeded from organisms already present in the virgin desert soil, but since no attempts had been made to isolate any of the organisms concerned, this assumption remained unverified.

In an attempt to verify previous conclusions, plantings of disease-free seed were again made in 1916 on lands never before planted to potatoes, and isolations of fungi were made from the soils. It is the purpose of this paper to set forth the results obtained from the plantings of 1916 and to report the fungi obtained from these isolations.

1916 PLANTINGS

In the spring of 1916 eight plots (No. 1–8) were planted with disease-free seed potatoes on irrigated land previously cropped with grain and alfalfa or clover, or with grain alone, but never with potatoes; one (No. 9) on irrigated virgin desert land never before in any crop; one (No. 10) on dry-farming land previously in grain but never in potatoes; and four (No. 11–14) on dry-farming land reclaimed from the desert in the spring of 1916 for the special purpose of planting these plots. Two of these plots (No. 7–8) were planted on the grounds of the experiment

[1] Reference is made by number (italic) to "Literature cited," p. 98–99.

station at Jerome, Idaho, on irrigated land never before planted with potatoes, but previously cropped with grain and alfalfa. These two plots consisted of a few hundred hills each. The remaining plots were planted in cooperation with farmers in southern Idaho, as follows: Plots 1 and 2 on irrigated, previously cultivated land near Blackfoot, Idaho; plot 3 on irrigated, previously cultivated land at Aberdeen, Idaho, on the grounds of the Aberdeen Experiment Station; plot 4 on irrigated alfalfa land, near Twin Falls, Idaho; plot 5 on irrigated alfalfa land near Murtaugh, Idaho; plot 6 on irrigated clover land near Jerome, Idaho; plot 9 on irrigated, virgin desert land, near Jerome, Idaho; plot 10 on dry-farming land previously cultivated but never irrigated, on the grounds of the Aberdeen Experiment Station, Aberdeen, Idaho; and plots 11 to 14 on virgin desert land not subject to irrigation, near Aberdeen, Idaho. Plots 1, 4, 5, 6, and 9 consisted of about 1 acre each; plot 2 of about 0.5 acre; plots 3 and 10 of about 0.2 acre each; and plots 11 to 14 of about 0.1 acre each.

The varieties planted in the test plots were as follows: Idaho Rural, Netted Gem, Carmen 3, Early Six Weeks, Irish Cobbler, People's, and Pearl. The seed tubers were selected in such a manner that no seed piece showing any internal or external evidence of disease was used. Every tuber was carefully examined in cutting, and the slightest discoloration was sufficient cause for discarding it. No dryrot-infected tubers were used, and all slight bruises were cut out to insure the freedom of the seed piece from any germs of disease which might be lurking in the bruised tissues. No tubers showing any evidence of common scab or the sclerotia of *Rhizoctonia solani* were employed. In addition to these precautionary measures, the seed tubers for plots 3, 7, 8, 10, 11, 12, 13, and 14, and the Netted Gem of plot 6, were selected from 1915 plantings from hills which showed no evidence of disease. The seed for the other plantings was selected from commercial stock.

All seed tubers used for the plots were disinfected after cutting for at least two hours in solutions of mercuric chlorid, strength 1:1,000, or stronger. Wherever planting machinery was employed, all parts of the machine were thoroughly scrubbed in a solution of mercuric chlorid (1:500). The seed for one plot at the Jerome Station (plot 7) was given special treatment as follows: The seed tubers were selected from disease-free hills in 1915. At planting time these tubers were taken to the laboratory and thoroughly scrubbed with a solution of mercuric chlorid (1:1,000) after which they were cut into seed pieces, only pieces showing clean white tissue being used. The seed pieces were then disinfected for two hours in a solution of mercuric chlorid (1:500) and carried to the field in the disinfecting solution, from which they were picked out by hand. They were then dropped into the row prepared to receive them and were immediately covered. It was believed that in this manner all

chance of contamination between the disinfecting bath and the field could be entirely removed.

During the growing season, inspections of each plot were made from time to time; but frequent frosts prevented an accurate determination of foliage and other diseases of the plants. Disease determinations were therefore reserved until harvest time. Those presented in this paper are confined to such diseases as were observed on the tubers when dug. At harvest time disease conditions were determined by digging 100 or more hills in different parts of each plot and examining the tubers from each hill separately. Disease percentages are expressed in terms of the percentage of hills infected with each disease and in the percentage of tubers infected. Vascular infection due principally to *Fusarium* spp. was the most abundant. Tuber-rots were occasionally found. Common scab and the sclerotia of *Rhizoctonia solani* were found on a small percentage of the tubers in most plots. The disease conditions observed in the several plots are given in Table I.

TABLE 1.—*Description of potato plots and percentages of disease observed at harvest*

Plot No.	Land reclaimed.	Previous cropping.	Character of farming.	Variety of potatoes.	Percentage of hills infected.				Percentage of diseased tubers (by weight).
					Vascular infection.	Tuber-rot.	Common scab.	Rhizoctonia.	
1	1909	1910–11, grain; 1912–1914, alfalfa: 1915, wheat.	Irrigation	People's	20	1	2	1	26.6
2	1908	1908–9, grain: 1910–11, fallow: 1912–1915, grain.	...do	...do	14	0	1	12	16.2
3	1909	1910–11, grain; 1912–1915, alfalfa.	...do	Netted Gem	25	1	0	1	10.1
				Idaho Rural	13	0	3	0	8.2
4	1907	1907, oats; 1908–1915, alfalfa.	...do	Idaho Rural	13	1	3	0	6.0
				Netted Gem	4	1	0	1	1.1
5	1908	1908, wheat; 1909–1915, alfalfa.	...do	Netted Gem	12	0	0	0	10.7
				Carmen 3	21	0	3	0	12.3
				Six Weeks	13	0	0	0	8.9
6	1910	1911–12, grain; 1913–1915, clover.	...do	Irish Cobbler	6	0	1	0	4.3
				Netted Gem	15	0	0	0	10.7
7	1910	1910, barley; 1911–1915, alfalfa.	...do	People's	16	4	1	0	19.0
				Idaho Rural	7	0	3	0	9.0
8	1910	See plot 7	...do	Idaho Rural	5	1	15	0	20.0
				Netted Gem	10	0	0	0	7.0
				People's	3	1	1	1	.....
				Six Weeks	20	2	14	0	22.4
9	1916	Virgin desert land	...do	Netted Gem	22	2	0	2	11.4
				People's	38	8	0	2	29.3
10	1912	1913, fallow; 1914, barley; 1915, fallow.	Dry farming.	Idaho Rural	34	0	6.7	0	48.0
				Netted Gem	37	2.5	0	0	39.0
11	1916	Virgin desert land	...do	Idaho Rural	20	0	0	0	8.0
				Netted Gem	15	0	0	0	7.0
				Pearl	10	0	1	0	4.0
12	1916	...do	...do	See plot 11	(a)	(a)	(a)	(a)	(a)
13	1916	...do	...do	...do	(a)	(a)	(a)	(a)	(a)
14	1916	...do	...do	...do	(a)	(a)	(a)	(a)	(a)

[a] No percentages determined.

In plots 12 to 14 no accurate estimate of the percentage of diseased plants or tubers could be arrived at. No rains fell on the plots after planting time and no previous cultivation to conserve the moisture had been given the land. The plants remained very small throughout the

season and very few produced any tubers. These three plots would not be mentioned in this report were it not for the fact that two tubers infected with common scab were found in plot 12 and that tubers showing infection of the vascular tissue were found in all three plots. The mycelium of *Rhizoctonia solani* was found on plant stems in each of the plots but the sclerotia were not found on any of the tubers. Isolations made in the laboratory from the discolored vascular tissue of tubers from each of these three plots showed the presence of several species of fungi, including *Fusarium radicicola*. A record of these plantings is therefore included because of the additional proof afforded of the presence in desert soils of parasitic fungi. Similar conditions also existed in plot 11, but better care was given this plot during the growing season and a better crop was obtained, rendering it possible to secure more accurate and extensive data.

When the plots were dug, infected tubers were collected from each and taken to the laboratory, where isolations were made. Isolations from discolored vascular tissues gave a variety of results: some remained sterile, some gave fungi which could not be associated in any way with the disease in question, a few gave pure cultures of *Fusarium radicicola*, and others gave this fungus associated with other fungi and bacteria. *F. radicicola* was isolated from the vascular tissue of tubers from each of the plots. Isolations from rotted tubers gave similar results, *F. radicicola* being the organism most frequently isolated. No attempt has been made to determine the organism associated with the common scab observed on the product from these plots. The scab is, however, similar in appearance to the common scab (*Actinomyces chromogenus* Gasperini) of the East, and it is assumed that it is caused by the same or a similar organism.

The average percentage of disease found present in the plots was as follows:

On irrigated, previously cultivated land (plots 1 to 8), percentage of hills infected: Vascular infection, 12.7 per cent; tuber-rot, 0.7 per cent; common scab, 2.7 per cent; Rhizoctonia or russet scab, 1 per cent; percentage of tubers diseased, all diseases, by weight, 12.03 per cent.

For the two varieties of plot 9, on irrigated, virgin desert land, percentage of hills infected: Vascular infection, 30 per cent; tuber-rot, 5 per cent; common scab, 0 per cent; Rhizoctonia or russet scab, 2 per cent; percentage of tubers infected, all diseases, by weight, 20.3 per cent.

For the two varieties of plot 10, on dry-farming land, previously cultivated, percentage of hills infected: Vascular infection, 35.5 per cent; tuber-rot, 1.25 per cent; common scab, 3.3 per cent; Rhizoctonia or russet scab, 0 per cent; percentage of tubers infected, all diseases, by weight, 43.5 per cent.

For the three varieties of plot 11, on dry-farming land, virgin desert soil, percentage of hills infected: Vascular infection, 15 per cent; tuber-

rot, 0 per cent; common scab, 0.33 per cent; Rhizoctonia or russet scab, 0 per cent; percentage of tubers infected, all diseases, by weight, 6.33 per cent.

In 1915 the averages for similar plots (10) were as follows:

Plots on irrigated, previously cultivated land: Vascular infection, 26 per cent; tuber-rot, about 0.5 per cent; common scab, 4.7 per cent; and Rhizoctonia or russet scab, about 2.8 per cent.

On virgin desert land, irrigated: Vascular infection, 29.3 per cent; tuber-rot, 5.6 per cent; common scab, 9.3 per cent; and Rhizoctonia or russet scab, 11.3 per cent.

No plots were planted on dry-farming land in 1915. In detail, the percentages of disease observed in the 1915 plantings are shown in Table II.

TABLE II.—*Percentages of diseases of potatoes observed in the plantings in 1915*

Plot No.	Reclaimed.	Previous cropping.	Variety of potato.	Percentage of hills infected.			
				Common scab.	Rhizoctonia.	Tuber-rot.	Vascular infection.
		AT JEROME STATION.					
1	1910	1910, barley; 1911–1914, alfalfa.	Idaho Rural	0	0	3.00	36
			....do	1.00	0	0	40
			Netted Gem	0	0	0	10
			Rural New Yorker	2.00	0	2.00	21
			Pearl	0	0	1.00	14
			People's	0	0	0	15
			Red Peachblow	0	0	0	20
			Burbank	0	0	0	5
		Average		0.37	0	0.77	20
		IN COOPERATION WITH IDAHO FARMERS.					
2	1907	1907, wheat; 1908–1914, alfalfa.	Carmen 3	30	8	0	46
3	1907	....do	Netted Gem	0	0	0	37
4	1908	1908–1915, orchard land, cropped with alfalfa and beans.	....do	0	6	0	44
5	1907	1907, grain; 1908–1914, alfalfa.	....do	0	1	1	2
6	1908	1908, grain; 1909–1913, idle; 1914, grain.	....do	0	0	0	5
7	?	Alfalfa, long period of years.	Red Peachblow	2	5	0	20
8	?	....do	Netted Gem	0	1	0	20
9	?	....do	Idaho Rural	10	4	0	41
10	1915	Raw desert land	....do	38	44	6	32
11	1915	....do	Netted Gem	0	5	10	53
12	1915	....do	....do	2	12	1	19
13	1915	....do	Idaho Rural	1	1	1	10
14	1915	....do	....do	10	7	5	29
15	1915	....do	People's	5	1	11	33

It will be seen that the percentages were much lower in 1916 than in 1915, and in this connection it is interesting to note that the prevailing temperature for the season of 1916 was much lower than for 1915. The temperature during the summer of 1915 was extremely high, while that of the season of 1916 was unusually low. It will be observed that, as in 1915, the percentage of infection was lower in the plots planted on irrigated, previously cultivated land than in the plots planted on virgin desert land.

ISOLATION OF FUNGI FROM THE SOIL

During the 1916 season 109 cultures were made from the soil, either in the field directly from the soil itself or in the laboratory from soil samples collected in the field. In making the soil cultures, whether in the field or in the laboratory, only sterile instruments, containers, and media were employed, and every possible precaution was taken to guard against air and other contaminations. It is believed that all such dangers were reduced to a minimum. In all, 58 soil cultures were made in the laboratory from five soil samples, and 51 in the field directly from the soil. Hereinafter the designation "soil sample" will be used in referring to the several sources of the cultures made in the laboratory from soil samples, and the words "soil group" in referring to the cultures made in the field directly from the soil. The soils employed are described as follows:

SOIL SAMPLE 1.—From a rather heavy clay loam on the grounds of the Jerome Experiment Station, at Jerome, Idaho. The land was reclaimed in 1910, planted to barley, and seeded with alfalfa, remaining in alfalfa until the spring of 1916, when it was plowed and put in condition for planting potatoes. The sample was taken on June 7, prior to the planting of the potatoes.

SOIL SAMPLE 2.—From the same source as soil sample 1, and taken a few feet distant. Sample taken on June 7.

SOIL SAMPLE 3.—From a desert soil from which the desert plants have just been removed and the soil put in condition for potato planting. Located just south of the city of Jerome, Idaho. The soil was a sandy-clay loam, reclaimed in May, 1916. The sample was taken on June 20 from a portion of the field which had not been planted. Plot 9, of the 1916 experimental plot, planted with disease-free seed potatoes, was planted in this field.

SOIL SAMPLE 4.—From a desert soil, supporting a typical growth of sagebrush and desert grasses, located about 25 miles northwest of Aberdeen, Idaho. The land had never been in cultivation and was situated nearly 20 miles from the nearest agricultural lands. Sample taken on August 18.

SOIL SAMPLE 5.—From the same type of soil as soil sample 4, located about 2 miles from the place where soil sample 4 was taken. Sample taken on August 18.

SOIL GROUP A.—From a very sandy soil in the Snake River Canyon, 15 miles southeast of Jerome, Idaho, and about 250 feet above the level of the river. This was a desert soil supporting only a very scant growth of desert grasses and dwarfed sagebrush plants. Cultures made on June 21.

SOIL GROUP B.—From a desert soil located about 11 miles southeast of Jerome, Idaho. The soil was principally of clay with a slight admixture of sand and was supporting a heavy growth of sagebrush and desert grasses. Cultures made on June 21.

SOIL GROUP C.—From the same soil as samples 1 and 2, on the grounds of the Jerome Experiment Station. The cultures were made on June 23, from soil in a portion of the field which had not been planted to potatoes.

SOIL GROUP D.—From the same soil as sample 4. Cultures made on August 18.

In taking the soil samples and in making the soil cultures, the writer was assisted by Mr. George L. Zundel,[1] each worker being present at the taking of each soil sample and in making each original soil culture. Mr. Zundel also gave valuable assistance in the examination and identification of the fungi obtained from the cultures, each worker verifying the observations of the other so far as possible.

On account of the necessity of closing the work early in the season, very little attention was given to the cultures made from samples 4 and 5, or to the cultures of soil groups B and D. For the same reason, a number of fungus forms which appeared in the remaining cultures were neglected, and undoubtedly many fungi were lost in transfer. The fungi reported in this paper therefore do not represent nearly the number of forms which appeared, but merely those which there was time to properly isolate and identify. In Table III is given a list of the fungi isolated and identified, showing the number of times each fungus form was isolated from each soil sample or group of soil cultures.

TABLE III.—*List of fungi isolated from the soil*

Name of fungus.	Number of times isolated.	Number of times isolated from each soil sample or group of soil cultures.								
		Group.				Sample.				
		A	B	C	D	1	2	3	4	5
Aspergillus sp	1	...	...	...	...	...	...	1	...	...
Absidia spinosa Lender	1	...	...	...	...	1	...	...	...	...
Absidia glauca Hagem	2	...	...	1	...	...	1	...	...	...
Absidia sp	2	...	...	...	...	1	...	1	...	...
Chaetomella sp	2	...	1	...	...	...	1	...	...	...
Fusarium affine Faut and Lamb	1	...	...	...	...	...	1	...	...	...
Fusarium dimerum Penz	1	...	...	...	...	1	...	...	...	...
Fusarium lanceolatum, n. sp	2	...	...	...	...	1	1	...	...	...
Fusarium acuminatum Ell. and Ev., emend. Wollenw	6	...	...	2	...	1	2	1	...	...
Fusarium sanguineum Sherb	2	...	...	...	...	...	...	2	...	...
Fusarium elegantum, n. sp	3	1	...	...	...	...	1	1	...	...
Fusarium idahoanum, n. sp	2	1	...	...	...	...	...	...	...	1
Fusarium trichothecioides Wollenw	2	...	...	...	...	...	...	2	...	...
Fusarium culmorum var. *leteius* Sherb	4	2	...	1	...	1	...	...	...	...
Fusarium discolor var. *triseptatum* Sherb	4	...	...	...	...	2	...	2	...	...
Fusarium subpallidum Sherb	2	2	...	...	...	...	...	...	...	...
Fusarium aridum, n. sp	1	...	1	...	...	...	...	...	...	...
Fusarium nigrum, n. sp	1	1	...	...	...	...	...	...	...	...
Fusarium radicicola Wollenw	9	1	...	1	...	1	2	4	...	...
Macrosporium commune Rabenh	2	...	...	...	...	1	1	...	...	...
Monascus sp	1	1	...	...	...	...	...	...	...	...
Mucor sphaerosporus Hagem	2	...	...	...	...	...	...	2	...	...
Mucor jansseni Lender	3	...	...	...	...	...	...	3	...	...

[1] Then Scientific Assistant, United States Departtment of Agriculture; now Assistant Professor of Biology, Brigham Young College, Logan, Utah.

TABLE III.—*List of fungi isolated from the soil*—Continued.

Name of fungus.	Number of times isolated.	Number of times isolated from each soil sample or group of soil cultures.								
		Group.				Sample.				
		A	B	C	D	1	2	3	4	5
Mucor spinescens Lender	1							1		
Mucor circinelloides Van Tieghem	1							1		
Mucor botryoides Lender	1							1		
Mucor plumbeus Bonorden	1						1			
Mucor sp. (undifferentiated)	9	1		5			2		1	
Penicillium strains, as arranged by Dr. Charles Thom and Miss M. B. Church:										
Penicillium viridicatum Westling (.503)	1			1						
(.712) Approximately *P. viridicatum* Westling	1		1							
(.452.3) A green Penicillium, suggestion of *P. pulvinum* Westling	1						1			
(.447) A strain of *P. expansum* Link	1						1			
(.480) Penicillium, blue-green series	1						1			
(.621) A blue-green Penicillium, fragrant	1	1								
(.500) Penicillium, coremiform series	1						1			
(.504) Penicillium, coremiform series	1							1		
(.508) Penicillium, coremiform series	1							1		
(.515) Penicillium, coremiform series	1							1		
The 4 preceding forms, not far from *P. expansum* Link.										
(.620) (.630) Penicillium, soil series (Cf. *P. humicola* Oudemans) tending toward coremiform production?	2	2								
(.401) Penicillium, soil series	1					1				
(.447) Penicillium, soil series	1						1			
(.451.1) Penicillium, soil series	1						1			
(.452.2) Penicillium, soil series	1						1			
(.507) Penicillium, soil series	1								1	
(.508) Penicillium, soil series	1								1	
(.604) Penicillium, soil series	1								1	
(.675) Penicillium, soil series	1	1								
(.750) Penicillium, soil series	1			1						
(.601) Penicillium, soil series, two strains mixed	1								1	
(.606) Penicillium, mixture, but chiefly soil series, admixture is coremiform	1								1	
Paecilomyces fusoideus Pers.	4	1	2					1		
Rhizopus nigricans Ehr.	1			1						
Rhizoctonia solani Kühn	5	2				1		2		
Stemphylium piriforme Bes.	3	3								
Stemphylium pusianum V. Szabo	2	1	1							
Trichoderma sp.	4			2		1	1			
Thamnidium elegans Link	2						1	1		
Torula sp.	4		1			1	1	1		
Verticillium sp. (.771)	1			1						
Verticillium sp. (.416)	2					2				
Verticillium sp. (.825)	3	1		1		1				
Verticillium sp. (.603)	1									1
Verticillium sp. (.477)	2					1		1		
Sterile mycelium, brown	1									1
Sterile mycelium, white	20	5		1	1	5	4	1		
Sterile mycelium, pink	3	2					1			
Sterile mycelium, yellow	1									1
Sterile mycelium, buff-colored, producing brownish sclerotia-like bodies	1	1								

DETAILED CONSIDERATION OF THE FUNGI ISOLATED

ASPERGILLUS

One strain of Aspergillus was isolated once from sample 3. This was identified by Dr. Thom and Miss Church (see p. 93).

ABSIDIA

Three species of the genus Absidia were identified as follows: *A. glauca* Hagem, isolated once from sample 2 and once from group C; *A. spinosa* Lender, isolated once from sample 1; and one unidentified species, isolated once each from samples 1 and 3. These were identified by Mr. Zundel

CHAETOMELLA

One species of the genus Chaetomella was twice identified, once each from group B and sample 2. This species is briefly described as follows: Mycelium white to gray or darker; perithecia separate, scattered on the mycelium but without distinct subicle, ovate-globose, 50 to 125 μ in diameter, black, beset with straight or curved bristle-like black hairs, 100 to 250 μ long, about 7 μ in diameter, septate, the septa distinguishable with difficulty; spores elliptical, olivaceous, 8 to 15 by 4 to 8 μ; character of sporophore uncertain.

FUSARIUM

Fourteen species of the genus Fusarium were isolated and identified. Because of the economic importance of this genus very careful attention was given each form isolated and in their identification, except where otherwise indicated in the following pages, authentic cultures were available for comparison. The writer is indebted to Mr. L. L. Harter, of this Office, for cultures of *Fusarium dimerum* Penz., *F. subpallidum* Sherb., *F. acuminatum* Ell. and Ev., emend Wollenw. *F. discolor* var. *triseptatum* Sherb., and *F. sanguineum* Sherb., as well as for many other cultures used in the comparisons, and to Dr. H. A. Edson, of this Office, for cultures of several strains of Fusarium which, it was thought, might be identical with certain of those isolated. Five of the strains isolated apparently differed from all species heretofore described and are herein presented as new species. The species isolated are as follows:

Section Dimerum [1]

Fusarium affine Faut and Lamb (*4, p. 68; 13, v. 14, p. 1125; 14, p. 126*).

The organism isolated agreed very closely with the original description given by Fautrey and Lambotte (*4*) and appeared to be very similar, both in microscopic characters and in habit of growth, to the strain

[1] The arrangement and grouping is after the plan suggested by Wollenweber (*20*).

studied by Sherbakoff (*14, p. 126*) though no authentic culture was available for comparison.

Habitat: In tubers and stems of *Solanum tuberosum* and in greenhouse soil, New York (*14, p. 126*). Isolated once from Idaho soils from sample 2.

Fusarium dimerum Penz (*1, p. 37, fig. 2–3; 8, p. 484; 12, p. 566; 13, v. 4, p. 704; 14, p. 127; 22, drawings 85–91*).

The organism isolated differed slightly from the original description by Penzig (*8, p. 484*), but the differences were not considered important enough to justify the establishment of a new species. On comparing it with a strain isolated from potatoes by Sherbakoff (*14, p. 127*), it was found to be identical, except that the conidia were slightly broader and longer and the growth on most media somewhat more rapid. In comparison with drawings of *F. dimerum* made by Wollenweber (*22*), the conidia appeared to be identical with those of several European strains of the organism. The organism isolated is briefly described as follows: Conidia lunar, slightly pedicillate, typically 1-septate, often 0-septate, sometimes 2-septate, very rarely 3-septate (only one 3-septate conidium was observed), borne singly on the mycelium, forming, on most media, a more or less continuous slimy layer, from slightly hyalin to light cinnamon buff,[1] on string-bean agar and steamed-potato media, a tufted growth from white to pink and cinnamon buff. The conidial measurements are as follows:

0-septate, 10 to 17 by 2.5 to 3.8 μ.
1-septate, 11 to 20 by 2.5 to 4.5 μ.
2-septate, 12 to 20 by 3 to 4.6 μ.

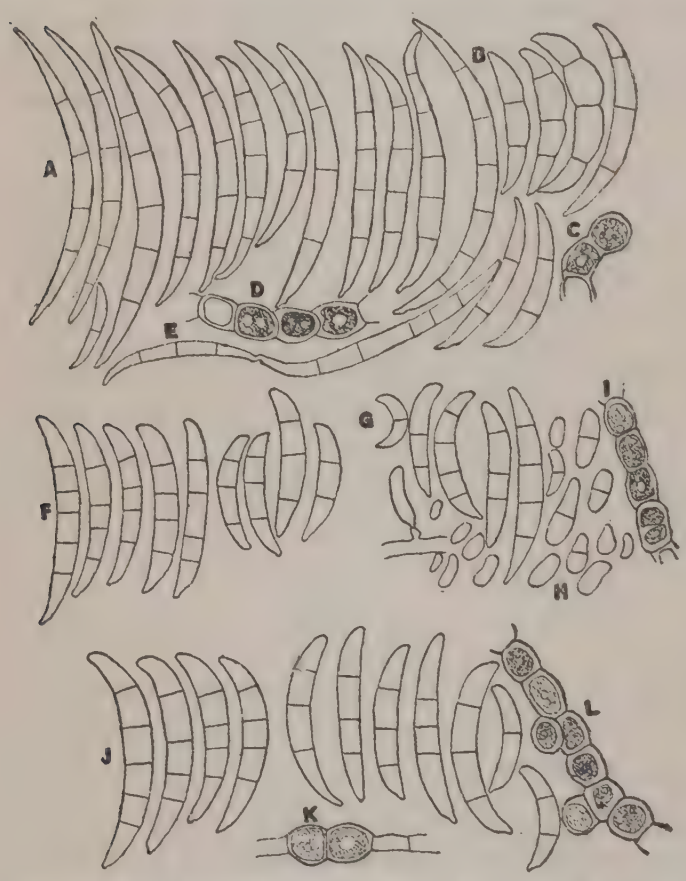

FIG. 1.—*A–E, Fusarium lanceolatum* n. sp. × 500: *A*, typical conidal forms sporodochia and pseudopionnotes; *B*, short conidia, some with swollen, barrel-like cells common on media containing an abundance of moisture; *C, D*, Chlamydospore in chains; *E*, Germinating conidium. *F–I, Fusarium elegantum*, n. sp. ×500: *F*, typical conidia from sporodochia; *G, H*, conidia from aerial mycelium; *G*, macroconidia; *H*, microconidia; *I*, chlamydospores in chains. *J–L, Fusarium nigum*, n. sp. ×500: *J*, typical conidia from sporodochia and aerial mycelium; *K, L*, chlamydospores in chains and groups.

[1] Colors given in this paper are according to RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

The average proportion of 1-septate conidia observed was 93 per cent; of 2-septate, 6 per cent; of 0-septate, 1 per cent (in mature cultures, 15 to 30 days old).

Habitat: On tubers and stems of *Solanum tuberosum* in Germany (*1*), and in Minnesota (*14*), on fruits of *Citrus medica* in Italy (*8*). Isolated once from Idaho soils from sample 1.

SECTION GIBBOSUM

Fusarium lanceolatum, n. sp. (Pl. A, 7, 8; fig. 1, A–E).

Conidia typically in pseudopionnotes, but also in aerial mycelium and sporodochia from nearly straight to strongly curved, usually distinctly pedicillate, typically 3-, 4-, and 5-septate, 6- and 7-septate common, higher septations rare, the 3-septate averaging 34 by 3.5 (22 to 52 by 2.5 to 5) μ, the 4-septate, 40 by 3.8 (22 to 60 by 2.8 to 5) μ, and the 5-septate, 48 by 4.1 (36 to 70 by 2.8 to 5.7) μ; aerial mycelium scantily developed, white when present, to dark maroon when well filled with conidia; sporodochia and pseudopionnotes ochraceous-orange at first, becoming dark maroon, often brighter shades of red to Brazil red; substratum on steamed potato often a bright orange, yellow modification on rice becoming brown with age; chlamydospores singly and in chains.

Habitat: Isolated twice from Idaho soils, once each from samples 1 and 2. When inoculated into potato tubers, no decay resulted.

The conidial measurements on various media are as follows:

FROM PSEUDOPIONNOTES ON STEAMED-POTATO PLUG, CULTURE 43 DAYS OLD

1-septate rare, 20 by 3 μ, only a few measured.
3-septate 23 per cent, 37 by 3.6 (28 to 50 by 3 to 4.3) μ.
4-septate 30 per cent, 48 by 4.2 (34 to 57 by 3 to 5) μ.
5-septate 44 per cent, 53 by 4.4 (43 to 68 by 3 to 5.7) μ.
6-septate 3 per cent, 62 by 4.8 (50 to 72 by 4.3 to 5) μ.
7-septate rare, 65 by 4.5 μ.

FROM PSEUDOPIONNOTES ON STEAMED MELILOTUS STEM, CULTURE 56 DAYS OLD

1-septate 2 per cent, 19 by 3 (14 to 27 by 2.4 to 3.5) μ.
2-septate 2 per cent, 20 by 3 (14 to 33 by 2.4 to 3.6) μ.
3-septate 20 per cent, 32 by 3 (26 to 48 by 2.5 to 3.8) μ.
4-septate 22 per cent, 38 by 3.3 (28 to 60 by 2.8 to 4.8) μ.
5-septate 42 per cent, 48 by 4 (38 to 64 by 3 to 5) μ.
6-septate 10 per cent, 58 by 4 (44 to 70 by 3 to 5) μ.
7-septate 2 per cent, 64 by 4.2 (56 to 72 by 3.5 to 5) μ.

FROM PSEUDOPIONNOTES ON POTATO AGAR (10 PER CENT GLUCOSE), CULTURE 60 DAYS OLD

1-septate 0.4 per cent, 18 by 3 (14 to 24 by 2.5 to 3.5) μ.
2-septate 1.5 per cent, 19 by 3 (15 to 26 by 2.5 to 3.8) μ.
3-septate 20.0 per cent, 33 by 3.4 (22 to 52 by 2.8 to 4) μ.
4-septate 20.5 per cent, 37 by 3.5 (28 to 60 by 3 to 4) μ.
5-septate 57 per cent, 47 by 3.5 (38 to 70 by 2.8 to 4) μ.
6-septate 0.2 per cent, 56 by 3.5 (42 to 72 by 3 to 4.2) μ.
7-septate 0.4 per cent, 60 by 3.6 (42 to 76 by 3 to 4.2) μ.

FROM AERIAL MYCELIUM ON STRING-BEAN AGAR, CULTURE 65 DAYS OLD

1-septate rare.
2-septate 2 per cent, 22 by 3 (20 to 36 by 2.5 to 3.2) μ.
3-septate 30 per cent, 36 by 4 (22 to 44 by 3 to 5) μ.
4-septate 11 per cent, 37 by 4.2 (22 to 48 by 3 to 5) μ.
5-septate 55 per cent, 44 by 4.5 (40 to 64 by 3 to 5.3) μ.
6-septate 1 per cent, 52 by 4.5 (42 to 68 by 3 to 5.4) μ.
7-septate 1 per cent, 56 by 4.5 (42 to 70 by 3 to 5.3) μ.
8- and 9-septate rare.

SUMMARY AND AVERAGE OF THE PRECEDING MEASUREMENTS

1 septate up to 2 per cent, 19 by 3 (14 to 27 by 2.4 to 3.5) μ.
2-septate up to 2 per cent, 20 by 3 (14 to 36 by 2.4 to 3.8) μ.
3-septate up to 30 per cent, 34 by 3.5 (22 to 52 by 2.5 to 5) μ.
4-septate up to 30 per cent, 40 by 3.8 (22 to 60 by 2.8 to 5) μ.
5-septate up to 57 per cent, 48 by 4.1 (36 to 70 by 2.8 to 5.7) μ.
6-septate up to 10 per cent, 61 by 4.2 (42 to 72 by 3 to 5.4) μ.
Higher septations rare.

SECTION ROSEUM

Fusarium acuminatum Ell. and Ev., emend. Wollenw. (*3, p. 441; 13, v, 14, p. 1125–1126; 14, p. 142; 21, p. 269–270, pl. 16, fig. G; 22, drawings 166, 168, 170*).

The strain isolated agreed very closely with a culture of the organism furnished by Dr. L. L. Harter. Conidial measurements and habits of growth on various media were very similar.

Habitat:

On partly decayed plants, especially on stems, roots, and tubers, also on fruits' Found on Solanum, Ipomoea, Fagus (beechnuts), and *Impatiens balsamina* in the United States of America (*21, p. 269*).

Isolated six times from Idaho soils, twice from group C, once from sample 1, twice from sample 2, and once from sample 3. This organism was once isolated from old roots of alfalfa which had been cut back in harvesting.

SECTION FERRUGINOSUM

Fusarium sanguineum Sherb. (*14, p. 193–196; pl. 3, fig. 7–8; pl. 6, fig. 1; 22, drawing 165*).

Identified by comparison with a strain of the organism isolated from potatoes by Sherbakoff (culture furnished by Dr. L. L. Harter).

Habitat:

On rotted tubers of *Solanum tuberosum* in association with *F. lutulatum* var. *zonatum*, Ithaca, N. Y. (*14, p. 194*).

Isolated twice from Idaho soils, from sample 3.

SECTION ELEGANS

Fusarium elegantum, n. sp. (Pl. A, 5, 6; fig. 1, F–I).

Microconidia usually present in aerial mycelium elliptical to oval, or slightly curved, typically 0-septate, often 1-septate, the 0-septate averaging 7.5 by 2.8 (4.5 to 11 by 2 to 4.5) μ, the 1-septate averaging

12 by 3.2 (10 to 17 by 2.8 to 4.5) μ; macroconidia in aerial mycelium, pseudopionnotes, and sporodochia, slightly curved, typically broader at the middle and in the upper half of their length, somewhat abruptly constricted toward the apex, slightly pedicillate, typically 3- and 4-septate, the 3-septate averaging 29 by 4.2 (19 to 41 by 3.5 to 5.5) μ, the 4-septate averaging 33 by 4.6 (26 to 46 by 3.6 to 5.7) μ, sometimes 2-septate, 1- and 5-septate rare; aerial mycelium typically well developed, white; sporodochia and pseudopionnotes, light salmon-orange to salmon-orange on most media, salmon-orange to old rose on Irish potato agar with 10 per cent of glucose; substratum but slightly discolored or not at all, slight-yellow modification on steamed rice; flesh colored to pinkish wartlike plectenchymic bodies often abundant, especially on steamed melilotus stems; sclerotia often present, dark blue (on steamed potato media); chlamydospores usually present in old cultures, but never abundant, intercalary in the mycelium.

Habitat: Isolated three times from Idaho soils as follows: Once each from samples 2 and 3 and once from group A. Attempts to induce decay in Irish potato tubers by inoculating with this organism were unsuccessful.

The conidial measurements on various media are as follows:

CONIDIA FROM SPORODOCHIA ON STEAMED MELILOTUS STEM, 19 DAYS OLD

0-septate 1 per cent (immature, about same as 3- and 4-septate).
2-septate 5 per cent, 18 by 4.3 (15 to 22 by 4 to 5) μ.
3-septate 50 per cent, 31 by 4.7 (23 to 36 by 3.5 to 5.5) μ.
4-septate 41 per cent, 35 by 5.2 (28 to 39 by 4.3 to 5.7) μ.
5-septate 3 per cent, 36 by 5.7 (28 to 40 by 4.2 to 5.7) μ.

CONIDIA FROM AERIAL MYCELIUM ON STEAMED MELILOTUS STEM, 21 DAYS OLD

Microconidia:
0-septate 14 per cent, 8 by 2.2 (7 to 11 by 2 to 2.8) μ.
1-septate 11 per cent, 13 by 3 (10 to 14 by 2.8 to 3.6) μ.
Macroconidia:
2-septate 3 per cent, 19 by 3.5 (18 to 21 by 2.8 to 3.6) μ.
3-septate 68 per cent, 28 by 4.2 (21 to 32 by 3.6 to 4.5) μ.
4-septate 4 per cent, 32 by 4.5 (28 to 36 by 4.3 to 5) μ.
5-septate rare (only one observed) 35 by 5 μ.

CONIDIA FROM SPORODOCHIA ON STEAMED POTATO-TUBER PLUG, 49 DAYS OLD

2-septate 1 per cent, 19 by 4.2 (16 to 22 by 4.3 to 5) μ.
3-septate 51 per cent, 30 by 4.3 (21 to 35 by 3.8 to 5) μ.
4-septate 48 per cent, 33 by 4.5 (26 to 41 by 4 to 5) μ.
5-septate rare (only one observed) 34 by 5.1 μ.

CONIDIA FROM PSEUDOPIONNOTES ON STEAMED POTATO-TUBER PLUG, 20 DAYS OLD

0-septate 1 per cent, immature.
1-septate rare, 20 by 4 (19 to 26 by 3.5 to 4.5) μ.
2-septate 1 per cent, 22 by 4.5 (20 to 26 by 3.8 to 5.0) μ.
3-septate 68 per cent, 30 by 4.3 (21 to 36 by 3.6 to 5) μ.
4-septate 30 per cent, 33 by 4.6 (29 to 41 by 3.6 to 5.7) μ.

CONIDIA FROM SPORODOCHIA ON STRING-BEAN AGAR, CULTURE 25 DAYS OLD

3-septate 77 per cent, 34 by 4 (26 to 41 by 3.8 to 4.8) μ.
4-septate 23 per cent, 34 by 4.2 (30 to 46 by 3.8 to 5) μ.
5-septate rare, only two observed, averaging 41 by 4.5 μ.

CONIDIA FROM AERIAL MYCELIUM ON POTATO AGAR, WITH 10 PER CENT OF GLUCOSE ADDED, CULTURE 26 DAYS OLD

Microconidia:
0-septate 84 per cent, 7 by 3.5 (4.5 to 11 by 2.5 to 4.5) μ.
1-septate 7 per cent, 11 by 3.5 (10 to 17 by 3 to 4.5) μ.
Macroconidia:
2-septate 1 per cent, 23 by 3.8 (19 to 26 by 3.4 to 4.5) μ.
3-septate 8 per cent, 26 by 4 (19 to 29 by 3.8 to 5) μ.
4-septate rare.

The conidia are rarely normal on this medium. Conidia from sporodochia and pseudopionnotes are usually swollen, with barrel-shaped cells. The sporodochia, often converging into a pseudopionnotal layer, soon become overgrown with mycelium containing a very high percentage of microspores.

SUMMARY AND AVERAGE OF THE FOREGOING MEASUREMENTS

Microconidia:
0-septate up to 84 per cent, 7.5 by 2.8 (4.5 to 11 by 2 to 4.5) μ.
1-septate up to 11 per cent, 12 by 3.2 (10 to 17 by 2.8 to 4.5) μ.
Macroconidia:
0-septate considered immature.
1-septate rare, 20 by 4 (19 to 26 by 3.5 to 4.5) μ.
2-septate up to 5 per cent, 20 by 4 (15 to 26 by 3.4 to 5) μ.
3-septate up to 68 per cent, 29 by 4.2 (19 to 41 by 3.5 to 5.5) μ.
4-septate up to 48 per cent, 33.4 by 4.6 (26 to 46 by 3.6 to 5.7) μ.
5-septate rare, 35 by 5 (28 to 41 by 4.2 to 5.7) μ.

It will be seen from the description that this organism lacks one of the typical characters of the section Elegans—namely, the characteristic vinaceous hues. This color reaction appears with this organism only on media rich in glucose but other characters are such that the writer feels justified in placing it in this section.

Fusarium idahoanum, n. sp. (Pl. B, 4–6; fig. 2, M–P.)

Microconidia always present in aerial mycelium and often in sporodochia and pseudopionnotes elliptical to oval, sometimes slightly curved, 0-septate, averaging 7 by 2.4 (4 to 12 by 1.5 to 3.5) μ; macroconidia slightly curved, typically gradually attenuated toward the apex, slightly pedicillate, typically 3-septate, averaging 25 by 4.1 (18 to 40 by 3 to 5) μ, 1-, 2-, and 4-septate common, 5-septate rare; aerial mycelium typically well developed, white at first, becoming pink to mallow-purple, often orange-pink when well filled with conidia, frequently developing shades of yellow on Irish potato agar with 10 per cent glucose; sporodochia and pseudopionnotes, light orange to orange; substratum (steamed rice) yellow to shades of brown, sometimes, in places, shades of pink to vina-

ceous, on Irish potato agar with 10 per cent of glucose, a rich amber-brown.

Habitat: Isolated twice from Idaho soils, once each from sample 5 and group A. When inoculated into Irish potato tubers, a very slight decay resulted. The organism was recovered from the decayed tissue; however, the decay was so slight that it is probable that the organism has no great parasitic faculty.

The measurements for the conidia on various media are as follows:

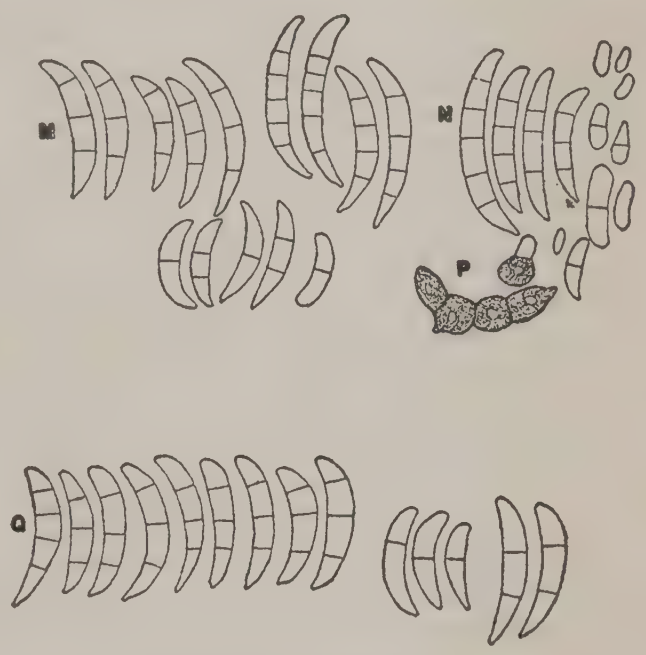

FIG. 2.—*M–P*, *Fusarium idahoanum*, n. sp. × 500: *M*, conidia from sporodochia; *N*, *O*, conidia from aerial mycelium; *N*, macroconidia: *O*; microconidia, 0– and 1–septate; *P*, chlamydospores formed in conidia. *Q*, *Fusarium aridum*, n. sp.: Typical conidial forms from sporodochia and aerial mycelium. ×500.

CONIDIA FROM SPORODOCHIA ON STEAMED POTATO PLUG, CULTURE 47 DAYS OLD

Microconidia:
- 0-septate rare, 6 by 1.8 μ, only a few measured.

Macroconidia:
- 1-septate 7 per cent, 16 by 3 (11 to 19 by 2 to 3.4) μ.
- 2-septate 19 per cent, 18 by 3.3 (12 to 19 by 2.6 to 3.4) μ.
- 3-septate 72 per cent, 24 by 4 (18 to 34 by 3 to 4) 9μ.
- 4-septate 2 per cent, 30 by 4.4 (26 to 34 by 3.8 to 5.3) μ.
- 5-septate rare, 29 by 4 μ.

CONIDIA FROM AERIAL MYCELIUM ON IRISH POTATO AGAR, WITH 10 PER CENT OF GLUCOSE, CULTURE 48 DAYS OLD

Microconidia:
- 0-septate 21 per cent, 7 by 2.5 (4 to 11 by 1.5 to 3.4) μ.

Macroconidia:
- 1-septate 9 per cent, 14 by 3.3 (11 to 17 by 2.2 to 3.7) μ.
- 2-septate 7 per cent, 16 by 3.3 (13 to 19 by 2.5 to 3.8) μ.
- 3-septate 56 per cent, 26 by 4 (19 to 38 by 3.5 to 4.9) μ.
- 4-septate 6 per cent, 30 by 4.3 (24 to 39 by 3.8 to 5.1) μ.
- 5-septate 1 per cent, 34 by 4.3 (30 to 39 by 3.8 to 5) μ.

CONIDIA FROM PSEUDOPIONNOTES ON STEAMED MELILOTUS STEM, CULTURE 57 DAYS OLD

Microconidia:
- 0-septate 16 per cent, 6 by 2.2 (5 to 11 by 1.8 to 3.5) μ.

Macroconidia:
- 1-septate 7 per cent, 14 by 3.2 (9 to 16 by 2.4 to 3.6) μ.
- 2-septate 6 per cent, 17 by 3.4 (12 to 21 by 2.6 to 3.8) μ.
- 3-septate 64 per cent, 28 by 4 (18 to 40 by 3.5 to 5) μ.
- 4-septate 7 per cent, 30 by 4.2 (22 to 40 by 3.5 to 5) μ.
- 5-septate rare.

CONIDIA FROM AERIAL MYCELIUM ON STRING-BEAN AGAR, CULTURE 61 DAYS OLD

Microconidia:
0-septate 12 per cent, 8 by 2.5 (6 to 12 by 1.5 to 3) μ.
Macroconidia:
1-septate 15 per cent, 13 by 3 (10 to 18 by 2.8 to 3.6) μ.
2-septate 8 per cent, 18 by 3.3 (14 to 21 by 3 to 4) μ.
3-septate 61 per cent, 24 by 4.4 (19 to 37 by 3.5 to 5) μ.
4-septate 3 per cent, 29 by 4.5 (19 to 42 by 3.5 to 5.2) μ.
5-septate 1 per cent, 30 by 4.6 (26 to 43 by 4 to 5.2) μ.

SUMMARY AND AVERAGE OF THE FOREGOING MEASUREMENTS

Microconidia:
0-septate up to 21 per cent, 7 by 2.4 (4 to 12 by 1.5 to 3.5) μ.
Macroconidia:
1-septate up to 15 per cent, 14.7 by 3.1 (9 to 19 by 2 to 3.7) μ.
2-septate up to 19 per cent, 17 by 3.3 (12 to 21 by 2.5 to 4) μ.
3-septate up to 72 per cent, 25.5 by 4.1 (18 to 40 by 3 to 5) μ.
4-septate up to 7 per cent, 29.7 by 4.4 (19 to 42 by 3.5 to 5.2) μ.
5-septate up to 1 per cent, 32 by 4.4 (26 to 43 by 3.8 to 5.2) μ.

Section Discolor

(2, 5, 6, 9, 18, 19, p. 206; 22, drawing 305).

Fusarium trichothecioides Wollenw.

The organism isolated agreed in every particular with the original description given by Wollenweber (*5*). On comparing it with a strain of *F. trichothecioides* used by the writer in previous experiments it was found to be identical. The organism isolated from the soil was inoculated into potato tubers and a typical powdery-dryrot produced.

Habitat: First reported in 1912 by Jamieson and Wollenweber (*5*) on potato tubers from Western States and demonstrated to be the cause of a dryrot of western potatoes and capable of attacking growing plants. In 1913 a species of Fusarium, called "*Fusarium tuberivorum* Wilcox and Link," was reported as causing a dryrot of potatoes in Nebraska by Wilcox, Link, and Pool (*18*). This fungus was demonstrated by Wollenweber (*19, p. 206*) and by Carpenter (*2*) to be identical with *F. trichothecioides* Wollenw., which opinion was later concurred in by one of the authors of *F. tuberivorum* (*6*), who demonstrated it to be a cause of potato-wilt. Investigations on the part of the writer failed to prove it a wilt-producing organism under ordinary field conditions in Idaho, but demonstrated it to be a prevalent cause of storage-dryrot in that State (*9*). Isolated twice from Idaho soils from sample 3.

Fusarium culmorum var. **leteius** Sherb. (*14, p. 242–244, pl. 4, fig. 1, 2, 10; pl. 5, fig. 9, text fig. 1D₂, 43*):

No authentic culture of the original strain of this organism was available for comparison, but the conidial dimensions and growth characters were so similar to those of the original description given by Sherbakoff

(*14, p. 242–244*) that there was no doubt in the mind of the writer as to its identity.

Habitat: On rotted tubers of *Solanum tuberosum* in New York (*14, p. 242*). Isolated four times from Idaho soils as follows: Twice from group A and once each from group C and sample 1.

Fusarium discolor var. triseptatum Sherb. (*14, p. 239–240, pl. 4, fig. 5–6; pl. 5, fig. 10; text fig. 1W$_1$, 42; 22, drawings 319, 320*).

The strain isolated differed from an authentic culture of the species (furnished by Dr. L. L. Harter) by having slightly smaller conidia. Otherwise the organism isolated appeared to be identical with that originally described by Sherbakoff (*14, p. 239–240*). In his Fusaria Autographice Delineata, Wollenweber (*22*) considers *F. discolor* var. *triseptatum* a synonym of *F. discolor* Ap. and Wollenw., which in turn he makes a synonym of *F. sambucinum* Fuch.

Habitat: On rotted tubers of *Solanum tuberosum*, together with *F. coeruleum*, Long Island, N. Y. (*14, p. 239*). Isolated four times from Idaho soils, twice each from samples 1 and 3

Fusarium subpallidum Sherb. (*14, p. 230–234; pl. 5, fig. 12, text fig. 39; 22, drawing 326*).

The form isolated was identified by comparing with a culture of the original strain (culture furnished by Dr. L. L. Harter), with which it was found to be identical in every important particular.

Habitat: On superficial dryrot of potato tubers from Louisiana (*14*). Isolated twice from Idaho soils from group A.

Fusarium aridum, n. sp. (Pl. B, 1-3; fig. 2, Q).

Conidia in aerial mycelium, pseudopionnotes, and sporodochia, slightly curved, typically broader in the upper half of their length, usually suddenly constricted at the apex, slightly pedicillate, typically 3-septate, averaging 27 by 4.2 (18 to 36 by 3 to 5) μ; 1, 2, and 4-septate usually present, the 4-septate rare; aerial mycelium typically well developed, white at first, becoming pink to vinaceous; substratum on steamed-potato plug, often vinaceous to Vandyke red; on Irish potato agar with 10 per cent of glucose vinaceous-purple to carmen; steamed rice shades of yellow and brown; sporodochia and pseudopionnotes, salmon-orange to light-orange. Chlamydospores not observed.

Habitat: Isolated once from Idaho soils, from group B. When inoculated into potato tubers, a slight decay resulted, which bore a close resemblance to the type of decay produced by *F. trichothecioides*. The decay proceeded downward into the tuber from the point of inoculation for about ⅛ inch during the first 10 days, but though kept at temperatures varying from 5° to 35° C. for several weeks, failed to proceed farther. The writer therefore does not consider it capable alone of causing extensive rotting of potato tubers.

Conidial measurements on various media are as follows:

FROM AERIAL MYCELIUM ON STEAMED MELILOTUS STEM, CULTURE 23 DAYS OLD

1-septate 1 per cent, 19 by 3.5 (17 to 23 by 3.4 to 3.8) μ.
2-septate 2 per cent, 19 by 3.8 (17 to 25 by 3.4 to 4) μ.
3-septate 97 per cent, 27 by 4 (22 to 34 by 3.5 to 4.3) μ.

FROM AERIAL MYCELIUM ON STEAMED-POTATO PLUG, CULTURE 63 DAYS OLD

1-septate 1 per cent, 18.5 by 3.8 (14 to 25 by 3.5 to 4) μ.
2-septate 5 per cent, 23 by 4.1 (17 to 28 by 3.5 to 4.5) μ.
3-septate 94 per cent, 25 by 4.3 (22 to 32 by 3.5 to 5) μ.
4-septate rare, only one observed.

FROM PSEUDOPIONNOTES ON STEAMED-POTATO PLUG, CULTURE 64 DAYS OLD

1-septate 1.5 per cent, 19 by 3.8 (17 to 22 by 3.5 to 4) μ.
2-septate 2.5 per cent, 22 by 4.2 (19 to 26 by 3.8 to 4.5) μ.
3-septate 96 per cent, 28 by 4.3 (21 to 32 by 3.8 to 4.5) μ.

FROM SPOROTOCHIA ON IRISH POTATO AGAR WITH 10 PER CENT OF GLUCOSE, CULTURE 51 DAYS OLD

1-septate 3.5 per cent, 16 by 3.2 (12 to 26 by 2.5 to 4) μ.
2-septate 10.5 per cent, 20 by 3.9 (14 to 30 by 2.8 to 4.5) μ.
3-septate 86 per cent, 26 by 4.2 (19 to 33 by 3 to 4.8) μ.

FROM SPOROTOCHIA ON STRING-BEAN AGAR, CULTURE 60 DAYS OLD

1-septate rare, only a few measured, 18 by 3 μ.
2-septate 2 per cent, 22 by 4 (16 to 29 by 2.8 to 4.5) μ.
3-septate 98 per cent, 29 by 4.5 (18 to 36 by 3 to 5) μ.
4-septate rare.

SUMMARY OF THESE MEASUREMENTS

1-septate up to 3.5 per cent, 18 by 3.5 (12 to 26 by 2.5 to 4) μ.
2-septate up to 10.5 per cent, 21 by 4 (14 to 30 by 2.8 to 5) μ.
3-septate up to 98 per cent, 27 by 4.2 (18 to 36 by 3 to 5) μ.
4-septate rare.

Fusarium nigrum, n. sp. (Pl. A, 1–4; fig. 1, J–L).

Conidia in aerial mycelium, pseudopionnotes, and sporodochia, slightly curved, somewhat abruptly constricted toward the apex, typically broader at the middle and in the upper half of their length, typically 3- and 4-septate, the 3-septate averaging 27.5 by 4.7 (18 to 38 by 3.6 to 5.9) μ, the 4-septate averaging 31 by 5 (21 to 43 by 3.6 to 6) μ; aerial mycelium typically well developed, from white to reddish brown, often nearly ox-blood red, the appearance of shades of red and brown signalizing the development of chlamydospores, on Irish potato agar with 10 per cent of glucose, various shades of red and brown, discoloring the media from amber-brown to nearly black; sclerotia-like bodies, consisting of masses of mycelium, conidia, and chlamydospores, typically pres-

ent on starchy media and steamed melilotus stems, from ox-blood red to sepia-brown and black; sporodochia salmon-orange to ochraceous-orange and buckthorn-brown (on string-bean agar). Chlamydospores, terminal and intercalary, singly and in chains and groups.

Habitat: Isolated once from Idaho soils, from group A. This organism failed to produce a decay when inoculated in potato tubers.

The measurements of the conidia on various media are as follows:

FROM AERIAL MYCELIUM ON STEAMED-POTATO PLUG, CULTURE 52 DAYS OLD

1-septate rare.
2-septate 9 per cent, 20 by 3.9 (17 to 26 by 3.6 to 4.8) μ.
3-septate 42 per cent, 30 by 5 (18 to 36 by 3.6 to 5.9) μ.
4-septate 40 per cent, 36 by 5.5 (24 to 43 by 4.2 to 6) μ.
5-septate 9 per cent, 39 by 5.6 (27 to 46 by 4.2 to 6.1) μ.
6-septate rare.

FROM AERIAL MYCELIUM ON STEAMED-POTATO PLUG, CULTURE 11 DAYS OLD

1-septate 21 per cent, 20 by 4.2 (16 to 24 by 3.5 to 4.9) μ.
2-septate 2 per cent, 22 by 4.4 (17 to 25 by 3.5 to 5) μ.
3-septate 70 per cent, 26 by 4.8 (19 to 33 by 3.9 to 5.4) μ.
4-septate 5 per cent, 27 by 5 (21 to 38 by 4 to 5.6) μ.
5-septate 2 per cent, 33 by 5.3 (24 to 41 by 4.8 to 5.8) μ.
6-septate rare.

FROM SMALL SPORODOCHIA, IN AERIAL MYCELIUM, ON STEAMED MELILOTUS STEM, CULTURE 51 DAYS OLD

2-septate 2 per cent, 20 by 4.1 (16 to 23 by 3.3 to 4.6) μ.
3-septate 65 per cent, 26 by 4.4 (20 to 33 by 3.6 to 4.7) μ.
4-septate 33 per cent, 31 by 4.9 (23 to 38 by 3.9 to 5.5) μ.
5-septate rare, 36 by 5.1 μ.

FROM SPORODOCHIA ON STRING-BEAN AGAR, CULTURE 33 DAYS OLD

2-septate 4 per cent, 22 by 4.4 (17 to 25 by 3.8 to 4.7) μ.
3-septate 56 per cent, 28 by 4.6 (22 to 38 by 3.8 to 5.2) μ.
4-septate 37 per cent, 33 by 4.7 (23 to 41 by 3.8 to 6) μ.
5-septate 3 per cent, 34 by 5.1 (23 to 44 by 3.8 to 6) μ.

SUMMARY AND AVERAGE OF THESE MEASUREMENTS

1-septate up to 21 per cent, 20 by 4.2 (16 to 24 by 3.5 to 4.9) μ.
2-septate up to 9 per cent, 22.5 by 4.2 (16 to 25 by 3.3 to 5) μ.
3-septate up to 70 per cent, 27.5 by 4.7 (18 to 38 by 3.6 to 5.9) μ.
4-septate up to 40 per cent, 31.7 by 5 (21 to 43 by 3.6 to 6) μ.
5-septate up to 9 per cent, 35.5 by 5.2 (23 to 46 by 3.8 to 6.1) μ.

SECTION MARTIELLA

Fusarium radicicola Wollenw. (*2, 11, 14, p. 257-260, pl. 6, fig. 8, text fig. 47; 21, p. 257-258, pl. 16, fig. K; 22, drawing 423*).

The organism isolated appeared to be identical with that originally described by Wollenweber (*21*) and when inoculated into potato tubers a typical blackrot was produced.

Habitat: On partly decayed tubers and roots of plants, such as *Solanum tuberosum*, in Europe and America (collected by Wollenweber) and *Ipomoea batatas* in the United States of America (collected by Harter and Field (*21, p. 257*).

On partly decayed tubers and roots of plants. Cause of potato dry-rot and jelly-end rot. Identified from the following: *Ipomoea batatas* (collected by Mr. L. L. Harter); *Musa sapientum* (collected by Mr. S. F. Ashby, Jamaica, Porto Rico); soil (collected by Mr. F. C. Werkenthin, Austin, Tex.) (*2, p. 206*).,

On rotted tubers of *Solanum tuberosum*, in Oregon, Idaho, and California (*14, p. 258*).

Werkenthin (*17*) in 1916 reported it from the soils of Texas. It was reported in a previous paper by the writer (*11*) as occurring on the roots of *Populus deltoides* at Jerome, Idaho, and was demonstrated to be the cause of a blackrot of the Irish potato tuber in Idaho. While on a tour of southern Arizona in November, 1915, *F. radicicola* was isolated several times from decaying roots of Egyptian cotton (*Gossypium barbadense*) and alfalfa (*Medicago sativa*) plants growing on irrigated lands in the Salt River Valley, near Phoenix, Ariz. The fungus was there found associated with a form of the rootrot of cotton and alfalfa, and the conditions under which it was found suggested that it must have entered the plant roots from the soil. It was isolated nine times from Idaho soils as follows: Once each from sample 1 and groups A and C, twice from sample 2, and four times from sample 3.

MACROSPORIUM

Macrosporium commune Rabenh.

Identified once from sample 1 and once from sample 2. This species was also once identified from decaying roots of an opuntia growing in the desert near Jerome, Idaho.

MONASCUS

A single species of the genus Monascus was once isolated from group A. It is briefly described as follows: Mycelium, white to gray; perithecia abundant, globose, black, seated on compact mass of mycelium, 15 to 18 μ in diameter; asci, one, filling the perithecium, many-spored; spores, oval to elliptical, hyalin, minute, 3 to 5 by 1 to 2 μ. Fungus in mass on steamed melilotus stem, dark-gray to black.

MUCOR

Six species of the genus Mucor were identified, and nine other unidentified forms were isolated. These nine forms apparently differed from each other and from all other species described, but it is not certain that

the differences were sufficient to justify classifying them as separate species. The species identified are as follows:

Mucor sphaerosporus Hagem, isolated twice from sample 3.

Mucor jansseni Lender, isolated three times from sample 3.

Mucor spinescens Lender, isolated once from sample 3.

Mucor circinelloides Van Tieghem, isolated once from sample 3.

Mucor botryoides Lender, isolated once from sample 3

Mucor plumbeus Benorden, isolated once from sample 2.

Nine unidentified species of Mucor, one from group A, five from group C, two from sample 2, and one from sample 4.

The species of this genus were identified by Mr. Zundel.

PENICILLIUM

A large number of Penicillium appeared in the soil cultures, and as many of these were isolated as the time available would permit. The separation of these forms into distinct strains was accomplished by Mr. Zundel, who submitted them, with some others, to Dr. Charles Thom for identification. With reference to these cultures, the following statement was prepared by Dr. Charles Thom and Miss M. B. Church, of the Microbiological Laboratory, Bureau of Chemistry:

The problem of nomenclature in Penicillium is complicated by the occurrence of numerous strains with strictly asexual fruit production (conidia production only), which have no reliable structural differences, but may show markedly different reactions in culture. The study and comparison of such series collected by us, as well as those sent to us by students in widely separated localities, have led to the conclusion advanced in the study of the *Penicillium luteum-purpurogenum* series (15) and in the study of *Aspergillus niger* (16) that there are whole groups of such saprophytes genetically so related as to differ very little in morphology, but which differ quantitatively in their activities. In culture such strains may be readily kept separate by the contrast in color reactions produced in the nutrient media used, or by the shade of color in the conidial area. In other words, this particular strain kept in pure culture under fairly uniform conditions gives approximately the same picture in successive culture over a long period of time. The chemistry of these differences is thus far unsolved. Comparison of large numbers of forms, however, separates them into series with the same range of variation in structure and reactions which appear to differ only in quantity, not in kind.

The soil cultures sent us by Mr. George L. Zundel from Jerome, Idaho, contain some interesting series, which are represented in every group of soil cultures we have received. Not all of these forms are Penicillia.

One series, No. 370, 467, 473, and 649 are cultures of Trichoderma, a genus constantly occurring in soil and soil-polluted substances. No adequate study of these forms as they occur in America has yet been made, although we have collected a large number of them as the basis for such a study.

No. 500, 504, 508, 515, with apparently accidentally admixtures in a few other cultures, prove to be a coremiform Penicillium with affinities to *P. expansum* Link. Members of this series have been examined from widely different sources. They appear to be cosmopolitan and almost omnivorous. *P. expansun*, in one or more of its forms, is the characteristic coremiform species found in the rotting of apples in

storage. In this connection many studies of its activities have been made. No adequate comparative study has yet shown the nature of the differences observable between these strains. Until such study has been made the separation of this series into species or varieties would necessarily be valueless.

No. 363 and 712 appear to be close to *P. viridicatum* Westling. The original of Westling's description was found in Sweden on roots and moldy twigs. A closely related strain from soil has since been sent us from England by Miss Dale, and similar strains from soil have come to us from widely separated States.

No. 502 is an Aspergillus for which we have yet no name to offer. This again appears in more than one series of soil cultures.

Among the very difficult Penicillia, No. 480 and 621 are probably identical members of the blue-green series; No. 452.3 suggests *P. pulitans* Westling. But by far the most interesting series in this collection is designated in Table III, the soil series, including No. 401, 447, 452.1, 597, 598, 601, 604, 606, 620, 630, 675, and 750. This lot of forms presents a series of characters which have come up repeatedly in studying

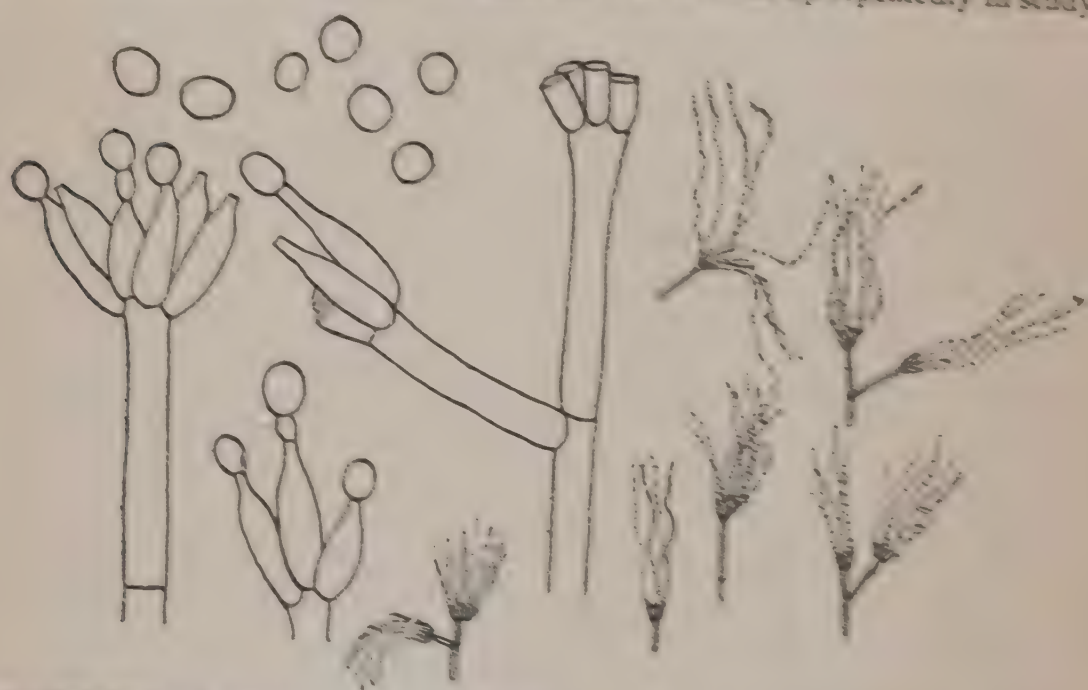

FIG. 3.—Penicillium soil series (strain 89): Colonies pale green, velvety at border, but more or less floccose in center with under side of mycelium rose to dark-red, conidia becoming globose, 2 to 3 μ in diameter. Drawn by Dr. Charles Thom.

the fungi of the soil. Similar forms have been received from Miss Dale, of England, from the soil bacteriologists of the United States Department of Agriculture, from Connecticut, and from New Jersey, and from other sources. Such forms seem to be found fairly constantly in soil cultures, but are not common in studies of foods and feeding stuffs.

Attempts to identify these forms by published descriptions were not satisfactory. Descriptions and drawings were prepared for a series of them, but these when critically compared demonstrated the close relationship of the organisms under consideration. It seemed very doubtful whether separation by such descriptions could be considered practical. The alternative is a group description drawn in broad enough terms to indicate the characteristic structure of the group with the range of variation observed. The following description is proposed:

Colonies in Czapek's solution agar white to gray, gray-green, pale-green, or pale bluish green, when old becoming various shades of gray and brown, spreading slowly but broadly with usually a wide sterile margin throughout the growing period and

slow development of colored fruit from the center outward, surface growth from velvety at the margin with center floccose to floccose out to the very edge of the colony, some strains zonate, reverse of colony at first colorless, in some strains remaining so, in others developing colors, a succession of colors appearing in series so that different strains become finally yellow, orange, orange-red, rosy, or even deep-red; the color mostly remains in the mycelium; hence, does not discolor the agar beyond the areas of immediate contact, if at all.

Odor produced, none or indefinite.

Conidiophores either rising directly from the substratum or as branches of aerial hyphæ, from very short up to 1,000 μ in length, or longer, slender mostly, 2 to 3 occasionally up to 4 μ in diameter, with walls smooth in some strains, slightly granular or roughened in others, or showing both conditions in the same culture; conidial fructifications variously branched from a single terminal verticil of sterigmata (conidiferous cells) to a verticil of metulæ (branches bearing verticils of sterigmata) including the main stalk prolonged and a single branch or a whorl of branches, more

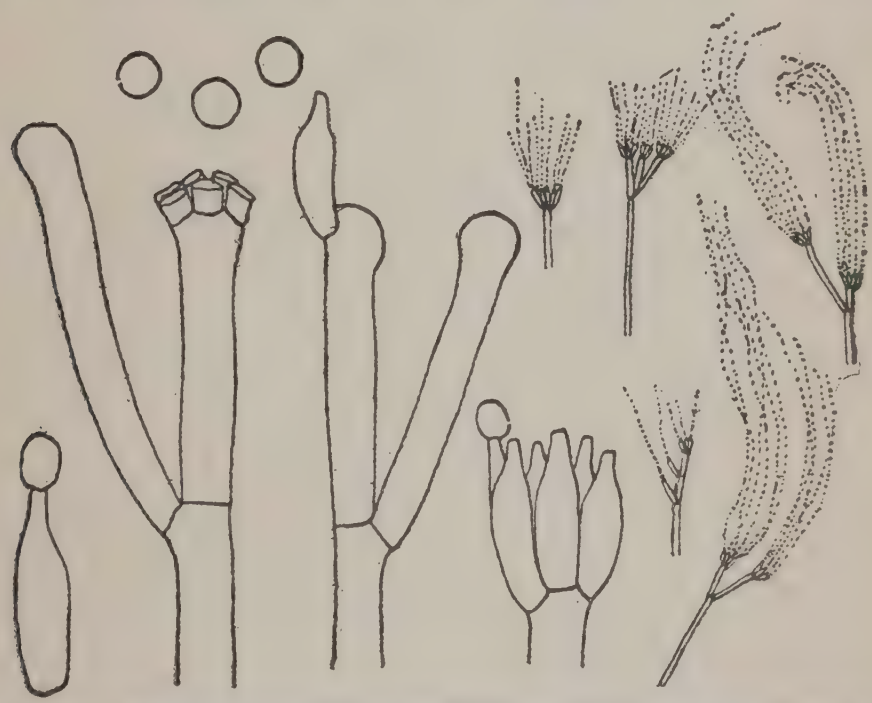

FIG. 4.—Penicillium soil series (strain 2490): Colonies differing very little in structure from strain 89 (fig. 3), but with reverse colors slowly yellow to orange. Drawn by Dr. Charles Thom.

rarely twice verticillate or partly so; sterigmata few in each verticil, mostly slender 7 to 10 by 2 to 2.5 μ, narrowing to a slender tube from which the conidia are formed. Conidia at first definitely elliptical, 1 to 2 by 2.5 μ, at first becoming 2.5 μ in diameter or even 3 to 3.5 μ in age, continuing elliptical or becoming almost globose, either smooth or delicately roughened or both conditions in the same strain (fig. 3, 4).

Habitat: Soils.

PERICONIA

Periconia byssoides Pers.

Isolated four times from Idaho soils, once from sample 3, once from group A, and twice from group B. Identified by Mr. Zundel.

RHIZOPUS

Rhizopus nigricans Ehr.

Isolated once from group C. Identified by Mr. Zundel.

RHIZOCTONIA

Rhizoctonia solani Kühn.

Identified five times from Idaho soils, twice from group A, once from sample 1, and twice from sample 3. Considerable difficulty was experienced in placing this organism in pure culture. This was accomplished but once, in one isolation from group A. The identification was made certain by a careful comparison with authentic cultures of *R. solani*. The other four identifications were made by a careful study of the mycelial and sclerotial characters, though the fungus was mixed in culture with other fungi, principally species of Fusarium.

Habitat: *R. solani* Kühn. has been reported from a great number of hosts, including the potato, from the soil and from decaying vegetable matter in the soil in humid regions. Cause of russet-scab of the potato and reported to be the cause of other potato disorders, including potato-rosette, aerial tubers, stemblight, damping-off of young potato plants, etc. Cause of damping-off of many economic plants. A complete host index is too long to be included here. A host index and historical sketch was published in 1916 in a work by Peltier (7), who isolated the organism from Illinois soils. The writer has identified the organism from the roots of alfalfa (*Medicago sativa*), sagebrush (*Artemesia tridentata*) and rabbit-brush (*Chrysothamnus graveolens*) in southern Idaho. Isolated five times from Idaho soils.

STEMPHYLIUM

Two species of the genus Stemphylium were isolated, as follows: *Stemphylium piriforme* Bos., isolated three times from group A; and *Stemphylium paxianum* V. Szabo., isolated once each from groups A and B. Identified by Mr. Zundel.

THAMNIDIUM

Thamnidium elegans Link., isolated once each from samples 2 and 3. Identified by Mr. Zundel.

TRICHODERMA

Four forms of the genus Trichoderma were isolated as follows: Twice from group C and once each from samples 1 and 2. (See statement by Dr. Thom, p. 93.) Identified by Dr. Charles Thom.

TORULA

One species of the genus Torula was isolated, once from group B and once each from samples 1, 2, and 3. This species is briefly described as follows:

Hyphæ dark, olivaceous, black in mass, septate; conidiophores simple; conidia in chains, unicellular, subglobose to ellipsoidal, olivaceous, black in mass, 8 to 15 by 6 to 8 μ.

VERTICILLIUM

Five species of the genus Verticillium were isolated, referred to in Table III by No. 419, 477, 603, 771, and 825. No. 419 was isolated twice from sample 1; No. 477 once each from samples 1 and 3; No. 603 once from sample 5; No. 771 once from group C; and No. 825 once each from sample 1 and groups A and C. No. 771 proved interesting in that it was once isolated from the discolored vascular bundles of a potato tuber grown in plot 11 (1916) on virgin desert soil, near Aberdeen, Idaho. This species is briefly described as follows: Conidia oval to elliptical, 2.5 to 8 by 1 to 3 μ, in chains on branches in verticillate whorls; mycelium and spores, in mass, from brick-red to dark reddish brown, on such media as steamed-potato plugs, steamed rice, and agars containing glucose.

STERILE MYCELIA

A sterile mycelium, reddish brown in color, was once isolated from sample 5, a sterile white form, apparently the same in each case was 20 times isolated, 8 times from sample 1, 4 times from sample 2, once from sample 3, 5 times from group A, and once each from each of groups C and D. A pink form was isolated once from sample 2 and twice from group A. At first it was thought that this pink form might be a species of Fusarium, which for some reason was slow in fruiting; but after nearly a year's growth in culture, no fruiting bodies were produced. A buff-colored form producing brownish to pinkish brown sclerotia-like bodies was once isolated from group A, and a yellowish form isolated once from sample 5.

SIGNIFICANCE OF THE INVESTIGATIONS

The special significance of these investigations lies in the finding of parasitic fungi in desert soils, on wild plants growing in the desert, and on cultivated plants in arid regions, where it is probable that the infection proceeded from the soil. These desert soils have commonly been supposed to support only a scant fungus flora and to be free from organisms of a parasitic nature. Their known presence in the soil, it is believed, explains the appearance of disease in the product of disease-free seed potatoes planted on new land and suggests that whenever it is attempted to grow disease-free potatoes, the rôle played by soil fungi must be taken into consideration.

SUMMARY

(1) Fungus forms were found to be abundant in desert soils.

(2) Three fungi, *Fusarium radicicola* Wollenw., *Fusarium trichothecioides* Wollenw., and *Rhizoctonia solani* Kühn., known to be parasitic on the Irish potato were isolated from Idaho soils never cropped with potatoes.

(3) Plantings of disease-free seed potatoes on new lands in southern Idaho failed to yield a disease-free product.

(4) The presence of parasitic fungi in these soils suggests that infection in potatoes may often originate with soil organisms.

(5) The results obtained from planting disease-free seed potatoes on cultivated lands never in potatoes, and on virgin desert land further substantiate the opinion that land, previously cropped with such crops as alfalfa, clover, and grain, is better adapted to the production of disease-free potatoes than virgin desert land.

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EXPLANATION OF PLATES A AND B

Plates A and B are reproductions of water-color drawings of cultures grown in the light at ordinary room temperatures, ranging from 16° to 26° C., in the laboratories of the U. S. Department of Agriculture at Washington, D. C. When grown under similar conditions in the arid West, the colors are often not so pronounced, owing probably to the earlier drying out of the media. It has also been noted that, when the cultures are grown at temperatures ranging from 30° to 42° C., the normal shades of red may be entirely wanting and in their stead dirty yellows and browns may appear. In text figures 1 and 2 attempt has been made to reproduce typical conidial and other spore forms by which the fungi might be identified at any stage of their growth on any of the media employed, such as steamed rice, steamed-potato plugs, steamed melilotus stems, Irish potato agar with 10 per cent of glucose added, and string-bean agar.

PLATE A

1–4.—*Fusarium nigrum*, n. sp.:

1. Culture 32 days old on steamed Irish potato plug.
2. Culture 40 days old on string-bean agar.
3. Culture 21 days old on steamed rice.
4. Culture 31 days old on Irish potato agar, plus 10 per cent of glucose. Note the nearly black color of the medium at the bottom of the tube, left-hand side.

5–6.—*Fusarium elegantum*, n. sp.:

5. Culture 25 days old on steamed rice.
6. Culture 18 days old on steamed-potato plug.

7–8.—*Fusarium lanceolatum*, n. sp.:

7. Culture 20 days old on steamed-potato plug.
8. Culture 18 days old on steamed rice.

1

2

3

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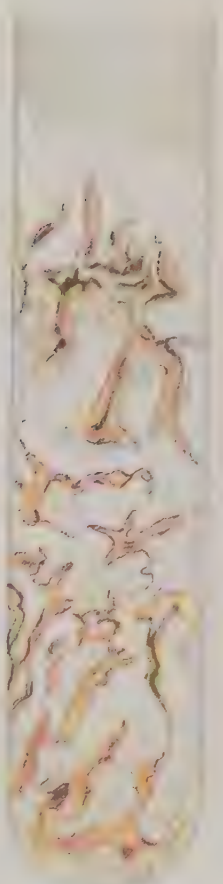

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A Stacy Hall

PLATE B

1–3.—*Fusarium aridum*, n. sp.:

1. Culture 40 days old on string-bean agar.
2. Culture 31 days old on steamed-potato plug.
3. Culture 17 days old on steamed rice.

4–6.—*Fusarium idahoanum*, n. sp.:

4. Culture 41 days old on Irish potato agar with 10 per cent of glucose added.
5. Culture 20 days old on steamed-potato plug.
6. Culture 21 days old on steamed rice.

1 2 3

4 5 6

O. Stacy Pratt

INVESTIGATIONS CONCERNING THE SOURCES AND CHANNELS OF INFECTION IN HOG CHOLERA

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INTRODUCTION

In hog cholera, as in other infectious diseases, the success of control measures is largely dependent upon a knowledge of the channels through which the infection is conveyed. Without such knowledge, or without the ability to close the avenues of transmission, if they are known, control by sanitation can not succeed. It is commonly known that hogs affected with cholera will transmit the disease to the nonimmunes which are allowed to associate with them. It is further known that pens or lots in which hogs sick of cholera have been kept are likely to harbor the infection, and that healthy hogs placed in such lots are liable to contract the disease. Many years ago, in a report issued by the Bureau of Animal Industry,[1] the sources and channels of infection were enumerated as follows:

(a) Pigs purchased from infected herds, or coming in contact with those from infected farms, or running over grounds occupied by diseased swine within two or three months.

(b) Infected streams may communicate the disease to herds below the source of infection.

(c) Virus may be carried in feed, implements, and on the feet and clothing of persons from infected herds and premises.

(d) Winds, insects, birds (particularly buzzards), and various animals may transport hog-cholera virus.

This statement of the ways in which hog cholera is spread forms the basis for our present-day sanitary regulations. It has seemed to the writers that perhaps in actual practice one or more of these channels of infection may be of preponderating importance, and that the determination of such a fact would greatly simplify the difficult problem of sanitary control. Although the experiments of the writers are not yet complete, sufficient data have been secured to make it desirable to render this report of progress, and it is hoped that others may undertake similar investigations. Conditions in nature are so variable that a single series of experiments can hardly yield results that would serve as a guide in practice in different localities under diverse climatic conditions.

[1] Hog cholera, its history, nature, and treatment. U. S. Dept. Agr. Dept. Rpt. 46, p. 123. 1899.

TRANSMISSION OF HOG CHOLERA BY PIGS

The fact that the blood and excreta and the eye secretions of hogs sick of cholera contain the virus of the disease is already well established. However, comparatively little systematic work has been done to ascertain whether the virus is discharged from the body only at certain stages or whether this occurs continuously throughout the course of the disease. Experiments on this point have been carried out in two groups. In the first, the pathogenic power of the blood, urine, feces, eye secretions, and nose secretions from hogs in different stages of the disease was determined (1) by the inoculation of susceptible pigs, (2) by feeding susceptible pigs with the secretions and excreta, and (3) by scattering the secretions and excreta in pens containing susceptible pigs. In the second group of experiments the contagiousness of the disease at different stages was determined by placing susceptible pigs in contact with those that were infected.

INFECTIOUSNESS OF THE BLOOD, EXCRETA, AND SECRETIONS OF CHOLERA-INFECTED PIGS

EXPERIMENT I.—Pig 1032x was injected subcutaneously with 5 c. c. of virus blood on October 11, 1916. This pig showed a rise of temperature on the fourth day, and the first visible symptoms were observed on the fifth day. The animal died on the tenth day, exhibiting well-marked hemorrhagic lesions at autopsy.

The clinical record of pig 1032x is given in Table I.

TABLE I.—*Record of pig 1032x*

Date.	Temperature.	Symptoms.
1916.	°F.	
October 11	.....	Normal. Injected this day.
October 13[a]	103.0	Normal.
October 12[a]	103.8	Do.
October 14[a]	103.8	Do.
October 15	104.9	Do.
October 16[a]	105.0	Sluggish.
October 17	105.8	Off feed.
October 18	107.2	Do.
October 19	107.0	Off feed; slight diarrhea.
October 20[a]	106.0	Off feed; profuse diarrhea.
October 21	.....	Dead.

[a] Blood, excreta, and secretions were tested on these dates.

On the first, second, third, fifth, and ninth days after injection the blood, urine, and feces, and the eye and nose secretions were collected and tested on susceptible pigs. Two pigs were injected with each material on each date, making a total of 10 pigs injected on each day.

The blood was obtained by bleeding from the tail. The urine and feces were collected in sterile bottles. The eye and nose secretions were

obtained on sterile cotton swabs, each swab being immediately rubbed up in 10 c. c. of sterile salt solution. The feces were shaken up in sterile salt solution, and the coarse particles were allowed to settle before injecting; the rather thick suspension was filtered through gauze and administered in doses of 10 c. c. The material was administered to the susceptible pigs in each instance by subcutaneous inoculation.

The results of this experiment are shown in detail in Table II.

This experiment shows that the blood and urine were virulent on the first day after injection, the feces on the second day, while the eye and nose secretions were virulent on the third day. As the pig which furnished the test materials did not develop fever until the fourth day and exhibited no visible symptoms until the fifth day, the experiment shows that the virus circulated in the body of the cholera-infected pig before the onset of fever and before the development of any visible symptoms. It is clear also that the virus was thrown off in the excreta and secretions during this time.

TABLE II.—*Results of Experiment I on infectiousness of blood, excreta, and secretions of infected pigs*

MATERIALS COLLECTED AND INJECTED ON OCTOBER 12, 1916 (FIRST DAY)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
1033x	40	5 c. c. of tail blood	Died October 28. Cholera lesions.
1034	55	do	Do.
[a]1035	45	5 c. c. of eye-swab dilution	Remained well.
[a]1036	65	do	Do.
[a]1037	45	5 c. c. of nose-swab dilution	Do.
[a]1038	70	do	Do.
1041	40	5 c. c. of urine	Sick October 20–24. Recovered.
1042	65	do	Do.
1039	40	10 c. c. of fecal suspension	Remained well.
1040	55	do	Do.

MATERIALS COLLECTED AND INJECTED ON OCTOBER 13, 1916 (SECOND DAY)

1043	40	5 c. c. of tail blood	Died October 30. Cholera lesions.
1044	55	do	Do.
[a]1051	35	5 c. c. of eye-swab dilution	Remained well.
[a]1052	65	do	Do.
[a]1049	40	5 c. c. of nose-swab dilution	Do.
[a]1050	55	do	Do.
1045	35	5 c. c. of urine	Killed in moribund condition November 14. Cholera lesions.
1046	60	do	Sick October 24 to November 17. Recovered.
1047	35	15 c. c. of fecal suspension	Killed in moribund condition November 14. Cholera lesions.
1048	55	do	Died November 12. Cholera lesions.

[a] These pigs were subsequently infected with virus blood to test their immunity and all developed typical hog cholera except 1038 and 1052. Pig 1038 suffered no ill effects from the virus infection and evidently possessed a high degree of natural immunity. Pig 1052 showed a temperature reaction following the virus injection but no visible symptoms.

TABLE II.—*Results of Experiment I on infectiousness of blood, excreta, and secretions of infected pigs*—Continued

MATERIALS COLLECTED AND INJECTED ON OCTOBER 14, 1916 (THIRD DAY)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
1053	40	5 c. c. of tail blood	Died October 27. Cholera lesions.
1054	70	.....do	Died October 30. Cholera lesions.
1055	40	5 c. c. of eye-swab dilution	Died November 7. Cholera lesions.
1056	65	.....do	Killed in moribund condition November 7. Cholera lesions.
[a]1057	45	5 c. c. of nose-swab dilution	Remained well.
1058	60	.....do	Sick October 23–30. Recovered.
1061	35	5 c. c. of urine	Died October 26. Cholera lesions.
1062	60	.....do	Died October 27. Cholera lesions.
1059	35	10 c. c. of fecal suspension	Died November 10. Cholera lesions.
1060	50	.....do	Killed in moribund condition November 10. Cholera lesions.

MATERIALS COLLECTED AND INJECTED ON OCTOBER 16, 1916 (FIFTH DAY)

1073	35	5 c. c. of tail blood	Died November 30. Cholera lesions.
1074	55	.....do	Sick October 21 to November 2. Recovered.
1071	35	5 c. c. of eye-swab dilution	Died November 3. Cholera lesions.
1072	60	.....do	Killed in moribund condition October 30. Cholera lesions.
1069	35	5 c. c. of nose-swab dilution	Died November 1. Cholera lesions.
1070	65	.....do	Sick October 21 to November 27. Recovered.
1065	35	5 c. c. of urine	Killed in moribund condition October 31. Cholera lesions.
1066	60	.....do	Died October 30. Cholera lesions.
1067	40	10 c. c. of fecal suspension	Killed in moribund condition October 31. Cholera lesions.
1068	65	.....do	Died October 26. Cholera lesions.

MATERIALS COLLECTED AND INJECTED ON OCTOBER 20, 1916 (NINTH DAY)

1081	30	2 c. c. of tail blood	Died October 31. Cholera lesions.
1082	55	.....do	Died October 30. Cholera lesions.
1085	30	5 c. c. of eye-swab dilution	Killed in moribund condition October 31. Cholera lesions.
1086	55	.....do	Do.
1083	50	5 c. c. of nose-swab dilution	Died November 7. Cholera lesions.
1084	30	.....do	Killed in moribund condition November 13. Cholera lesions.
1079	30	5 c. c. of urine	Do.
1080	45	.....do	Died November 6. Cholera lesions.
1087	50	10 c. c. of fecal suspension	Died October 26. Cholera lesions.
1088	25	.....do	Died October 27. Cholera lesions.

[a] This pig was subsequently injected with virus blood to test its immunity and developed typical hog cholera.

EXPERIMENT II.—In this experiment, which was begun on June 28, 1917, the eye and nose secretions, urine, and feces were collected from a cholera-infected pig (No. 427), which received an injection of 5 c. c.

of virus blood on June 25. This pig showed a fever temperature on June 28, was off feed on June 29, showed weakness and conjunctivitis on June 30, and died on July 11, showing well-marked hemorrhagic lesions at autopsy. The secretions and excreta were collected on the third, fifth, and seventh days after the virus injection—that is, on June 28, June 30, and July 2, 1917.

The eye and nose secretions were collected on sterile cotton swabs. The urine and feces were collected in wide-mouth sterile bottles, the excreta being collected as they were passed by the pig. The eye and nose swab dilutions were prepared by washing off the cotton swabs contaminated with the secretions in 60 c. c. of normal salt solution. The urine was diluted with an equal volume of salt solution. The fecal suspension used on the third and fifth days was prepared by shaking up approximately 100 gm. of feces in 300 c. c. of salt solution. On the seventh day 50 gm. of feces to 300 c. c. of salt solution was used. The secretions and excreta were tested on susceptible pigs within a few hours after they were collected.

On each of the days on which the secretions and excreta were collected, two susceptible pigs were injected with 5 c. c. each of an eye-swab dilution, two with 5 c. c. each of a nose-swab dilution, two with 5 c. c. each of urine, and two with 10 c. c. each of fecal suspension.

On the first day on which the secretions and excreta were collected, two pigs were fed with 25 c. c. each of eye-swab dilution, two with 25 c. c. each of nose-swab dilution, two with 5 c. c. each of urine, and two with 10 c. c. each of the fecal suspension. The materials were mixed with slop when fed. The same pigs were fed with the same doses on each of the succeeding days on which materials were collected. These pigs therefore received three feedings of supposedly infectious materials.

On each of the three days on which the secretions and excreta were collected 25 c. c. of a freshly prepared eye-swab dilution were scattered on the floor of a pen containing two susceptible pigs, while 25 c. c. of a freshly prepared nose-swab dilution were scattered in a second pen containing two pigs. In a third pen containing two susceptible pigs freshly collected urine was scattered as follows: On the third day 50 c. c., on the fifth day 140 c. c., and on the seventh day 130 c. c. In a fourth pen containing two pigs about 360 c. c. of freshly prepared fecal suspension were scattered on the third, fifth, and seventh days. The same pigs were thus exposed in a similar manner to the supposedly infectious materials on each of the days upon which these materials were collected.

The results obtained in this experiment were briefly as follows:

The eye and nose secretions and the feces collected on the third day were infectious when injected. The urine collected on this day was not infectious when injected.

The eye and nose secretions, the urine, and the feces collected on the fifth and seventh days were all infectious when injected.

The eye and nose secretions, the urine and the feces, collected on the third, fifth, and seventh days proved to be noninfectious when fed and when scattered in the pens.

The results of this experiment are shown in detail in Table III.

TABLE III.—*Results of Experiment II on the infectiousness of blood excreta, and secretions of infected pigs*

Pig No.	Weight.	Material injected.	Result.
		MATERIALS COLLECTED AND INJECTED ON JUNE 28, 1917 (THIRD DAY)	
	Pounds.		
444	55	5 c. c. of eye-swab dilution	Died July 16. Cholera lesions.
445	40	do	Killed in moribund condition July 16. Cholera lesions.
446	55	5 c. c. of nose-swab dilution	Died July 15. Cholera lesions.
447	40	do	Died July 19. Cholera lesions.
[a] 448	55	10 c. c. of diluted urine	Remained well.
[a] 449	50	do	Do.
450	30	10 c. c. of fecal suspension	Sick July 5 to 17; cholera symptoms. Recovered.
451	30	10 c. c. of fecal suspension	Died July 18. Cholera lesions.
		MATERIALS COLLECTED AND INJECTED ON JUNE 30, 1917 (FIFTH DAY)	
503	40	5 c. c. of eye-swab dilution	Killed in moribund condition July 19. Cholera lesions.
504	60	do	Do.
505	50	5 c. c. of nose-swab dilution	Killed in moribund condition July 23. Cholera lesions.
506	50	do	
507	40	10 c. c. of diluted urine	Died July 16. Cholera lesions.
508	65	do	Killed in moribund condition July 16. Cholera lesions.
509	40	10 c. c. of fecal suspension	Killed in emaciated condition July 26. Cholera lesions.
510	40	do	Killed in emaciated condition July 27. Cholera lesions.
		MATERIALS COLLECTED AND INJECTED ON JULY 2, 1917 (SEVENTH DAY)	
515	40	5 c. c. of eye-swab dilution	Killed in moribund condition July 19. Cholera lesions.
516	40	do	Do.
517	40	5 c. c. of nose-swab dilution	Killed in moribund condition July 20. Cholera lesions.
518	40	do	Do.
519	40	10 c. c. of diluted urine	Do.
520	50	do	Do.
521	45	10 c. c. of fecal suspension	Do.
522	45	do	Killed in moribund condition July 19. Cholera lesions.

[a] These pigs were subsequently exposed to hog cholera by injections of virus blood, and all proved to be susceptible.

TABLE III.—*Results of Experiment II on the infectiousness of blood excreta, and secretions of infected pigs*—Continued

MATERIALS COLLECTED AND FED ON JUNE 28, JUNE 30, AND JULY 2, 1917 (THIRD, FIFTH, AND SEVENTH DAYS)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
[a] 452	60	25 c. c. of eye-swab dilution...	Remained well.
[a] 453	55	.....do.....	Do.
[a] 454	40	25 c. c. of nose-swab dilution....	Do.
[a] 455	40	.....do.....	Do.
[a] 456	50	10 c. c. of diluted urine.....	Do.
[a] 457	45	.....do.....	Do.
[a] 458	60	10 c. c. of fecal suspension.....	Do.
[a] 459	45	.....do.....	Do.

MATERIALS COLLECTED AND SCATTERED IN PENS ON JUNE 28, JUNE 30, AND JULY 2, 1917 (THIRD, FIFTH, AND SEVENTH DAYS)

[a] 460	50	25 c. c. of eye-swab dilution...	Remained well.
[a] 461	60	.....do.....	Do.
[a] 462	45	25 c. c. of nose-swab dilution....	Do.
[a] 463	50	.....do.....	Do.
[a] 464	55	50 c. c., 140 c. c., and 130 c. c. of urine.	Do.
[a] 465	55	.....do.....	Do.
[a] 466	45	360 c. c. of fecal suspension....	Do.
[a] 467	45	.....do.....	Do.

[a] These pigs were subsequently exposed to hog cholera by injections of virous blood and all proved to be susceptible.

EXPERIMENT III.—In this experiment, which was begun on August 2, 1917, the blood, eye and nose secretions, urine, and feces were collected from a cholera-infected pig on the seventh day after injection. Pig 2226, which furnished the blood, secretions, and excreta, was injected with virus on July 26, 1917. This pig went off feed on July 30, showed conjunctivitis, diarrhea, and weakness on August 2, and died on August 3, exhibiting extensive hemorrhagic lesions at autopsy.

The blood was obtained by bleeding from the tail, and was defibrinated and strained through sterile gauze. The secretions and excreta were collected in the same manner as in Experiment II. The blood, secretions, and excreta were tested when fresh—that is, within a short time after they were collected, and were also tested after being held for 24 and 48 hours at room temperature (72° to 85° F.).

In collecting the eye secretions, three swabs were taken from the eyes. One of these, soon after collection, was rubbed up in 35 c. c. of normal salt solution and two pigs were injected with 15 c. c. each of the suspension. The two other swabs were held at room temperature. At the end of 24 hours a second swab was rubbed up in 35 c. c. of salt solution and two pigs injected with 15 c. c. each of the resulting suspension. At

the end of 48 hours the third swab was rubbed up in 35 c. c. of salt solution and two pigs injected, as before, with 15 c. c. each.

Three nose swabs were prepared and tested in the same manner as the eye swabs. The urine was used undiluted. In testing the feces, which were diarrheal in character, about 5.5 gm. of the feces were shaken up in 40 c. c. of normal salt solution and filtered through sterile gauze; susceptible pigs were given 15 c. c. each of this suspension.

After standing at room temperature for 24 hours, the blood had darkened but had a normal odor. The secretions on the eye and nose swabs had become dry. The urine had not changed in appearance The feces had become darker in color and drier.

After standing at room temperature for 48 hours, the blood was quite dark in color and had a putrid odor. The urine showed a sediment and had a stale odor. The feces were darker and had a putrid odor.

The results obtained in this experiment were briefly as follows:

When injected fresh, the blood, secretions, and excreta obtained on the seventh day were all infectious.

When injected after being held for 24 hours at room temperature, the blood, secretions, and excreta were still infectious.

When injected after being held for 48 hours at room temperature, the blood, urine, and feces remained infectious, but the eye and nose secretions were no longer infectious.

The detailed results of this experiment are shown in Table IV.

TABLE IV.—*Results of Experiment III on the infectiousness of blood, excreta, and secretions of infected pigs*

MATERIALS COLLECTED AND INJECTED ON AUGUST 2, 1917 (SEVENTH DAY)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
590	75	5 c. c. of tail blood	Died August 12. Cholera lesions.
591	75	do	Died August 16. Cholera lesions.
592	70	15 c. c. of eye-swab dilution	Do.
593	70	do	Killed in moribund condition August 20. Cholera lesions.
594	70	15 c. c. of nose-swab dilution	Died August 20. Cholera lesions.
595	70	do	Died August 10. Cholera lesions.
596	75	5 c. c. of urine	Died August 18. Cholera lesions.
597	75	do	Died August 19. Cholera lesions.
598	70	15 c. c. of fecal suspension	Died August 9. Cholera lesions.
599	70	do	Do.

TABLE IV.—*Results of Experiment III on the infectiousness of blood, excreta, and secretions of infected pigs*—Continued

MATERIALS COLLECTED ON AUGUST 2, 1917, AND INJECTED ON AUGUST 3, 1917 (HELD FOR 24 HOURS AT ROOM TEMPERATURE)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
600	70	2 c. c. of tail blood	Killed in moribund condition August 16. Cholera lesions.
601	70	.....do	Died August 15. Cholera lesions.
602	65	15 c. c. of eye-swab dilution	Died August 16. Cholera lesions.
603	65	.....do	Died August 19. Cholera lesions.
604	70	15 c. c. of nose-swab dilution	Killed in moribund condition August 20. Cholera lesions.
605	70	.....do	Do.
606	70	5 c. c. of urine	Died August 16. Cholera lesions.
607	70	.....do	Do.
608	70	15 c. c. of fecal suspension	Died August 11. Cholera lesions.
609	70	.....do	Died August 10. Cholera lesions.

MATERIALS COLLECTED AUGUST 2, 1917, AND INJECTED ON AUGUST 4, 1917 (HELD FOR 48 HOURS AT ROOM TEMPERATURE)

612	70	2 c. c. of tail blood	Died August 12. Cholera lesions.
613	70	.....do	Died August 13. Cholera lesions.
[a] 614	70	15 c. c. of eye-swab dilution	Remained well.
[a] 615	70	.....do	Do.
[a] 616	60	15 c. c. of nose-swab dilution	Do.
[a] 617	60	.....do	Do.
618	65	5 c. c. of urine	Died August 15. Cholera lesions.
619	65	.....do	Killed in moribund condition August 15. Cholera lesions.
620	70	15 c. c. of fecal suspension	Died August 11. Cholera lesions.
621	70	.....do	Do.

[a] These pigs were subsequently injected with virus blood to test their immunity, and as a result all developed hog cholera.

EXPERIMENT IV.—In this experiment, which was begun on September 21, 1917, the eye and nose secretions, urine, and feces of a pig infected by virus injection were collected on the second, third, fifth, and seventh days after injection. Pig 669, which furnished the secretions and excreta for this experiment, received an injection of virus blood on September 19. This pig showed a fever temperature on September 24, was off feed on September 26, developed diarrhea and purple skin, and died on October 3, showing extensive hemorrhagic lesions at autopsy.

The eye and nose secretions were collected on sterile cotton swabs, each of which was washed off in 25 c. c. of sterile normal salt solution. The urine and feces were collected in the same manner as in the preceding experiments. The urine was used undiluted. A suspension of the fecal matter was made with normal salt solution, using 5 gm. to 50 c. c. of the salt solution, which was then strained through sterile gauze. In all instances, unless otherwise stated, the materials were freshly prepared and used within a short time after they were collected.

On each of the days on which the above materials were collected, two pigs were injected with 10 c. c. each of the eye-swab dilution, two with 10 c. c. each of the nose-swab dilution, two with 5 c. c. each of urine, and two with 20 c. c. each of the fecal suspension.

On the first day on which materials were collected, two pigs were fed 10 c. c. each of the eye swab dilution, two were fed with 10 c. c. each of nose swab dilution, two with 5 c. c. each of urine, and two with a suspension of 2.5 gms. of fecal matter. The materials in each instance were fed in the slop. The same pigs were fed with the same doses on each of the succeeding days on which the materials were collected. These pigs, therefore, received four feedings of supposedly infectious materials.

On the first day on which materials were collected 20 c. c. of eye-swab dilution were scattered over the floor of a pen containing two pigs, 20 c. c. of nose-swab dilution were scattered in a second pen containing two pigs, 200 c. c. of urine were scattered in a third pen containing two pigs, and 30 gm. of feces suspended in salt solution were scattered in a fourth pen containing two pigs. The same pigs were exposed in a similar manner to similar amounts of supposedly infectious materials on each of the succeeding days upon which these materials were collected. This set of pigs was exposed, therefore, on four days to supposedly infectious materials scattered in their pens.

The materials collected on the fifth day were also held at room temperature, varying from 60° to 80° F., for 24 hours, and injected in the same doses as when injected fresh.

The materials collected on the seventh day were held at room temperature, varying from 60° to 75° F., for 24 and 48 hours, and injected in the same doses as when injected fresh. The eye- and nose-swab dilutions at the end of 48 hours remained neutral, the urine became slightly more acid, and the feces, which were neutral when collected, became slightly acid.

The results obtained in this experiment were briefly as follows:

The freshly collected secretions and excreta obtained on the second day proved to be noninfectious when injected.

The freshly collected secretions and excreta obtained on the third day were all infectious when injected, except the urine, which proved to be noninfectious.

The freshly collected secretions and excreta obtained on the fifth and seventh days were all infectious when injected.

The freshly collected secretions and excreta proved to be noninfectious when fed, with the possible exception of one pig fed with eye-swab dilution. This pig developed hog cholera 23 days after the date of last feeding.

The freshly collected secretions and excreta also proved to be noninfectious when scattered in the pens, with the exception of one pen

where the nasal secretion was scattered. In this pen one pig sickened 16 days after the date on which nasal secretion was last scattered in the pen.

The secretions and excreta obtained on the fifth day and held at room temperature for 24 hours were all infectious.

The secretions and excreta obtained on the seventh day and held at room temperature for 24 hours were all infectious.

In the case of the secretions and excreta obtained on the seventh day and held at room temperature for 48 hours, the urine and feces were infectious, while the eye and nose secretions proved to be no longer infectious.

The results of this experiment are shown in detail in Table V.

TABLE V.—*Results of Experiment IV on the infectiousness of blood, excreta, and secretions of infected pigs*

Pig No.	Weight.	Material injected.	Result.
MATERIALS COLLECTED AND INJECTED ON SEPTEMBER 21, 1917 (SECOND DAY)			
	Pounds.		
[a] 737	65	10 c. c. of eye-swab dilution...	Remained well.
[a] 738	50	.....do.....	Do.
[a] 739	55	10 c. c. of nose-swab dilution...	Do.
[a] 740	60	.....do.....	Do.
[a] 741	65	5 c. c. of urine.....	Do.
[a] 742	45	.....do.....	Do.
[a] 743	45	20 c. c. of fecal suspension.....	Do.
[a] 744	60	.....do.....	Do.
MATERIALS COLLECTED AND INJECTED ON SEPTEMBER 22, 1917 (THIRD DAY)			
761	55	10 c. c. of eye-swab dilution...	Died October 9. Cholera lesions.
762	55	.....do.....	Died October 12. Cholera lesions.
763	70	10 c. c. of nose-swab dilution...	Died October 9. Cholera lesions.
[a] 764	50	.....do.....	Remained well.
[a] 765	45	5 c. c. of urine.....	Do.
[a] 766	65	.....do.....	Do.
767	50	20 c. c. of fecal suspension.....	Died October 4. Cholera lesions.
768	60	.....do.....	Died October 12. Cholera lesions.
MATERIALS COLLECTED AND INJECTED SEPTEMBER 24, 1917 (FIFTH DAY)			
769	65	10 c. c. of eye-swab dilution...	Died October 12. Cholera lesions.
770	55	.....do.....	Do.
771	60	10 c. c. of nose-swab dilution..	Died October 10. Cholera lesions.
772	50	.....do.....	Died October 8. Cholera lesions.
773	50	5 c. c. of urine.....	Died October 12. Cholera lesions.
774	50	.....do.....	Killed in moribund condition October 12. Cholera lesions.
775	65	20 c. c. of fecal suspension.....	Died October 10. Cholera lesions.
776	50	.....do.....	Do.

[a] These pigs were subsequently exposed to hog cholera by virus injections to test their immunity and all developed cholera except pigs No. 764, which remained well and probably possessed considerable natural immunity.

TABLE V.—*Results of Experiment IV on the infectiousness of blood, excreta, and secretions of infected pigs*—Continued

MATERIALS COLLECTED AND INJECTED ON SEPTEMBER 26, 1917 (SEVENTH DAY)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
785	40	10 c. c. of eye-swab dilution	Died October 8. Cholera lesions.
786	40	.....do	Killed in emaciated condition November 12. Cholera lesions.
787	65	10 c. c. of nose-swab dilution	Died October 13. Cholera lesions.
788	40	.....do	Do.
789	65	5 c. c. of urine	Do.
790	45	.....do	Do.
791	70	20 c. c. of fecal suspension	Died October 11. Cholera lesions.
792	45	.....do	Do.

MATERIALS COLLECTED ON SEPTEMBER 24 (FIFTH DAY) AND INJECTED ON SEPTEMBER 25, 1917 (HELD FOR 24 HOURS AT ROOM TEMPERATURE)

777	30	10 c. c. of eye-swab dilution	Died November 5. Cholera lesions.
778	55	.....do	Sickened October 19. Developed chronic hog cholera.
779	40	10 c. c. of nose-swab dilution	Died October 25. Cholera lesions.
780	60	.....do	Died November 3. Cholera lesions.
781	45	5 c. c. of urine	Died October 12. Cholera lesions.
782	65	.....do	Do.
783	35	20 c. c. of fecal suspension	Died October 11. Cholera lesions.
784	50	.....do	Do.

MATERIALS COLLECTED ON SEPTEMBER 26 (SEVENTH DAY) AND INJECTED ON SEPTEMBER 27, 1917 (HELD FOR 24 HOURS AT ROOM TEMPERATURE)

793	60	10 c. c. of eye-swab dilution	Died October 17. Cholera lesions.
794	80	.....do	Do.
795	50	10 c. c. of nose-swab dilution	Killed in emaciated condition November 12. Cholera lesions.
796	70	.....do	Sickened, but recovered.
797	80	5 c. c. of urine	Died October 19. Cholera lesions.
798	60	.....do	Do.
799	45	20 c. c. of fecal suspension	Died October 14. Cholera lesions.
800	50	.....do	Developed chronic hog cholera.

MATERIALS COLLECTED ON SEPTEMBER 26 (SEVENTH DAY) AND INJECTED ON SEPTEMBER 28, 1917 (HELD FOR 48 HOURS AT ROOM TEMPERATURE)

[a] 801	60	10 c. c. of eye-swab dilution	Remained well.
[a] 802	65	.....do	Do.
[a] 803	60	10 c. c. of nose-swab dilution	Do.
[a] 804	60	.....do	Do.
805	65	5 c. c. of urine	Died October 16. Cholera lesions.
806	60	.....do	Do.
807	65	20 c. c. of fecal suspension	Do.
808	55	.....do	Died October 15. Cholera lesions.

[a] These pigs were subsequently exposed to hog cholera by virus injections to test their immunity and all developed cholera except pigs Nos. 803 and 804, which remained well and probably possessed considerable natural immunity.

TABLE V.—*Results of Experiment IV on the infectiousness of blood, excreta, and secretions of infected pigs*—Continued

MATERIALS COLLECTED AND FED ON SEPTEMBER 21, 22, 24, AND 26, 1917 (SECOND, THIRD, FIFTH, AND SEVENTH DAYS)

Pig No.	Weight.	Material injected.	Result.
	Pounds.		
745	60	10 c. c. of eye-swab dilution...	Contracted hog cholera from contact with pig 746.
746	45	.....do.....	Sickened October 19 and died October 29. Cholera lesions.
[a] 747	65	10 c. c. of nose-swab dilution...	Remained well.
[a] 748	50	.....do.....	Do.
[a] 749	70	5 c. c. urine.....	Do.
[a] 750	55	.....do.....	Do.
[a] 751	60	2.5 gm. of feces in salt solution..	Do.
[a] 752	45	.....do.....	Do.

MATERIALS COLLECTED AND SCATTERED IN PENS ON SEPTEMBER 21, 22, 24, AND 26, 1917 (SECOND, THIRD, FIFTH, AND SEVENTH DAYS)

[a] 753	60	20 c. c. of eye-swab dilution...	Remained well.
[a] 754	45	.....do.....	Do.
755	65	20 c. c. of nose-swab dilution...	Sickened October 12 and died October 27. Cholera lesions.
756	50	.....do.....	Remained well.
[a] 757	70	200 c. c. of urine.....	Do.
[a] 758	55	.....do.....	Do.
[a] 759	60	30 gm. of feces in salt solution..	Do.
[a] 760	50	.....do.....	Do.

[a] These pigs were subsequently exposed to hog cholera by virus injections to test their immunity and all developed cholera except pig No. 758, which remained well and probably possessed considerable natural immunity.

In the four experiments which have just been described the virus was found to be present in the circulating blood of the cholera-infected pig on the first, second, third, fifth, seventh, and ninth days after injection.

The experiments indicate that the urine may be infectious as early as the first day after injection. In one experiment the urine was found to be infectious from the first to the ninth days, while in two experiments it was not infectious on the third day but was infectious on the fifth and seventh days. From these results it appears that, while the virus may be present in the urine earlier than the fourth day, it is quite regularly present in the urine of cholera-infected pigs by the fourth or fifth days.

In one experiment the virus was present in the feces on the second day, while in another experiment it was absent on the second day. In three experiments the virus was present in the feces on the third day, and it would thus appear that virus is thrown off quite regularly in the feces by the second or third day.

In two experiments the virus was absent from the eye and nose secretions on the second day, while in three experiments it was present in these secretions on the third day, and it is worthy of note that on those days there was no abnormal discharge from either the eyes or nose. It

would thus appear that the virus is present quite regularly in the eye and nose secretions by the third day.

The foregoing results are summarized in Table VI.

TABLE VI.—*Summary of results obtained in Experiments I to IV from injection tests of blood, excreta, and secretions of cholera-infected pigs* [a]

Days after injection.	Blood.	Urine.	Feces.	Eye secretions.	Nose secretions.
EXPERIMENT I					
1	+	+	−	−	−
2	+	+	+	−	−
3	+	+	+	+	+
5	+	+	+	+	+
9	+	+	+	+	+
EXPERIMENT II					
3	Not tested	−	+	+	+
5	do	+	+	+	+
7	do	+	+	+	+
EXPERIMENT III					
7	+	+	+	+	+
EXPERIMENT IV					
2	Not tested	−	−	−	−
3	do	−	+	+	+
5	do	+	+	+	+
7	do	+	+	+	+

[a] + indicates that the material was virulent: the injected pigs sickened or died. − Indicates that the material was not virulent: the injected pigs remained well, and contracted cholera upon subsequent exposure.

In contrast with the foregoing results, which were obtained by injecting the various materials, the results obtained from feeding the same materials and from scattering the same in pens with susceptible pigs were strikingly different. When fed and when scattered in pens, the freshly collected secretions and excreta obtained on the second, third, fifth, and seventh days proved to be noninfectious, with the possible exception of two pigs. One pig which was fed with eye secretion developed cholera 23 days after the date of the last feeding, and one pig exposed to nose secretion scattered in the pen developed hog cholera 16 days after the material was last scattered in the pen. It thus appears that although the virus is undoubtedly thrown off in the secretions and excreta of the sick animal at an early stage of the disease and the disease may be surely and readily

conveyed by injection, susceptible pigs do not readily contract the disease when fed with the infectious secretions and excreta or when exposed to these materials scattered in pens. These experiments suggest that perhaps the virus in the eye and nose secretions may be more readily conveyed by feeding and scattering than the virus in the urine and feces.

Secretions and excreta collected on the fifth and seventh days and held at room temperature (60° to 80° F.) for 24 hours proved to be infectious when injected. In the case of secretions and excreta which were collected on the seventh day and held at room temperature (60° to 75° F.) for 48 hours before injection, the urine and feces proved to be infectious, but the eye and nose secretions were no longer so. It should be noted that the eye and nose secretions were preserved on swabs and had dried before the last tests of virulence were made.

Of the four cholera-infected pigs which furnished the secretions and excreta for these experiments, two showed the first visible symptoms of sickness on the fourth day after injection, one on the sixth day, and one on the seventh day. As the experiments show that the virus is present quite regularly in the eye and nose secretions and in the feces by the third day, and may be present in the blood and urine as early as the first day, it becomes at once apparent that a cholera-infected pig may be a source of danger before the animal shows any visible symptoms of disease. A subsequent experiment (No. V) proves this danger to be a real one and that infected pigs may transmit the disease by contact during the period of incubation and before the appearance of visible symptoms. In this connection the possibility suggests itself that mild, unrecognized cases of hog cholera may occur and that such cases may be a factor in the spread of hog cholera.

CONTAGIOUSNESS OF HOG CHOLERA AT DIFFERENT STAGES OF THE DISEASE

The object of the following experiments was to determine whether, by mere contact, infected pigs are capable of transmitting hog cholera at all stages of the disease or whether there are certain periods, early or late, when the disease is not contagious. As will be seen, an endeavor was made to reduce, as far as possible, the likelihood of infection through contaminated pen litter.

Experiment V.—The experiment was carried out in the following manner: Three pigs, No. 1101, 1102, and 1103, were injected subcutaneously with blood from a sick pig on November 1 and were at once placed in a clean, disinfected pen, together with two uninoculated, susceptible pigs. The injected pigs and the susceptible, exposed pigs were allowed to remain together for 48 hours, at the end of which time the injected pigs and the exposed pigs were transferred to separate, clean, disinfected pens. Two more susceptible pigs were then placed with the injected pigs in their clean pen for 48 hours. The injected and

the exposed pigs were again transferred to separate, clean, disinfected pens. This was repeated at 48-hour intervals up to and including the tenth day.

The records of the three pigs which furnished the exposure are given in Table VII.

TABLE VII.—*Records of the three pigs which served as the source of hog-cholera infection*

Date.	Pig 1101.		Pig 1102.		Pig 1103.	
	Temperature.	Symptoms.	Temperature.	Symptoms.	Temperature.	Symptoms.
1916.	°F.		°F.		°F.	
Nov. 1	.....	Normal. Injected this day.	.....	Normal. Injected this day.	.....	Normal. Injected this day.
Nov. 2	103.8	Normal.....	104.0	Normal.....	103.7	Normal.
Nov. 3[a]	103.3	.....do.....	103.6	.....do.....	103.6	Do.
Nov. 4	104.7	.....do.....	104.2	.....do.....	104.4	Do.
Nov. 5[a]	.....	.....do.....	.....	.....do.....	.....	Do.
Nov. 6	104.4	Off feed.....	104.2	Off feed.....	104.4	Off feed.
Nov. 7[a]	103.6	.....do.....	104.2	.....do.....	103.6	Do.
Nov. 8	106.0	.....do.....	105.2	.....do.....	106.0	Do.
Nov. 9[a]	104.8	.....do.....	104.0	.....do.....	104.5	Do.
Nov. 10	103.8	Off feed; diarrhea.	105.4	.....do.....	105.4	Do.
Nov. 11[a]	104.4	.....do.....	104.4	.....do.....	105.4	Off feed; conjunctivitis, weakness, and diarrhea.

[a] The pigs were transferred to clean, disinfected pens on these dates and 2 susceptible pigs placed with them for 48 hours.

The subsequent history of the pigs which furnished the exposure was as follows: Pig 1101 died on November 14 and exhibited extensive hemorrhagic lesions of hog cholera. Pig 1102 developed conjunctivitis and had a fever temperature up to November 17; this pig recovered and was first reported as normal on November 24. Pig 1103 died on November 15, and showed hemorrhagic lesions with ulceration of cecum and colon.

The results of the experiment are shown in Table VIII.

Only 2 of the 12 exposed pigs escaped infection, and those were the two which were exposed to the infected pigs during the first 48 hours after injection. All of the other exposed pigs developed acute hog cholera and either died or were killed when in a moribund condition. The injected pigs which furnished the exposure did not show visible symptoms of disease until the fifth day.

TABLE VIII.—*Results of experiment V on the contagiousness of hog cholera (1916)*

[All pigs were exposed for 48-hour periods by association with cholera-sick pigs 1101, 1102, and 1103]

Pig No.	Weight.	Date exposed to sick pigs.	Date of transfer to disinfected pen.	Result.
	Pounds.			
1104	65	Nov. 1	Nov. 3	Remained well.
1105	65	Nov. 1	Nov. 3	Do.
1114	40	Nov. 3	Nov. 5	Died November 19; hemorrhagic lesions.
1115	20	Nov. 3	Nov. 5	Killed in moribund condition, November 27; hemorrhagic lesions.
1124	85	Nov. 5	Nov. 7	Died November 29; hemorrhagic lesions.
1125	70	Nov. 5	Nov. 7	Killed December 1; in advanced stage of disease; extensive hemorrhagic lesions; one ulcer in cecum.
1126	65	Nov. 7	Nov. 9	Died November 19; extensive hemorrhagic lesions.
1127	65	Nov. 7	Nov. 9	Killed in moribund condition November 20; extensive hemorrhagic lesions and ulceration of cecum and colon.
1134	70	Nov. 9	Nov. 11	Died November 22; hemorrhagic lesions.
1135	70	Nov. 9	Nov. 11	Killed in moribund condition November 27; hemorrhagic lesions with ulceration of cecum.
1144	65	Nov. 11	Nov. 13	Died November 30; extensive hemorrhagic lesions.
1145	70	Nov. 11	Nov. 13	Died December 1; extensive hemorrhagic lesions.

This experiment serves to corroborate Experiments I to IV, and shows very clearly that cholera-infected pigs may transmit the disease by contact prior to the appearance of visible symptoms. This is undoubtedly one of the reasons why the disease spreads so rapidly throughout a susceptible herd, once the infection has been established.

Experiment I indicated that the blood and urine of an inoculated pig were infectious 24 hours after inoculation and that the blood, urine, and feces were all infectious after 48 hours; yet in Experiment V the disease was not transferred by contact exposure during that period. It is an interesting and perhaps significant fact that the time at which contagiousness develops as shown by Experiment V coincides with the appearance of the infection in the eye and nose secretions. The number of experiments here recorded are only sufficient to permit the suggestion that perhaps the eye and nose secretions play a very important rôle in the dissemination of hog cholera.

Experiments VI, VII, VIII, and IX were carried out with a view to determining whether hog cholera may be transmitted by contact in the later or more advanced stages of the disease, as well as in the early stages.

EXPERIMENT VI.—This experiment was designed to afford a comparison between contact infection alone and combined pen infection and contact infection.

Four pigs, No. 1097x, 1098x, 1099, and 1100, were injected, each with 5 c. c. of virus blood on November 1 and were placed in the same pen. All of these pigs showed first visible symptoms on the fifth day after inoculation and developed the usual cholera symptoms. It was the intention to remove two of these sick pigs to a clean, disinfected pen at the end of 14 days, when the pigs would be in the late stages of the disease, and to expose two susceptible pigs with them; and two susceptible pigs were to have been exposed with the two pigs remaining in the original, infected pen. Pig 1100, however, died on the tenth day, exhibiting extensive hemorrhagic lesions at autopsy, so the experiment had to be modified as follows:

On November 11, ten days after inoculation and seven days after the appearance of first visible symptoms, pig 1099 was transferred to a pen which had been cleaned and disinfected and the disinfectant subsequently removed by washing. On the same date two susceptible pigs, No. 1138, and 1139, were placed in the disinfected pen with the sick pig, No. 1099. At the time of transfer, pig 1099 showed a temperature of 105.6° F. and was recorded as off feed and showing weakness, conjunctivitis, and diarrhea; this pig died four days later, on November 15, and the autopsy revealed estensive hemorrhagic lesions and ulceration of the cecum and colon. The two susceptible pigs, No. 1138 and 1139, promptly contracted hog cholera from contact with the sick pig, showing first visible symptoms on November 16, five days after exposure, and were found dead on November 21; both showed extensive hemorrhagic lesions at autopsy.

On November 11, two susceptible pigs, No. 1142 and 1143, were placed with the two remaining sick pigs, No. 1097x and 1098x, which had been left in the original, infected pen. At this time pig 1097x showed a temperature of 106.2° F. and was recorded as off feed and as showing conjunctivitis, weakness, and diarrhea; pig 1098x showed a temperature of 106.4° and was recorded as off feed and showing conjunctivitis and weakness. Pigs 1097x and 1098x both died on November 14, and both showed extensive hemorrhagic lesions at autopsy. The two susceptible pigs, No. 1142 and 1143, promptly contracted hog cholera, showing first visible symptoms on November 16, five days after exposure; they were found dead on November 21, and both showed extensive hemorrhagic lesions at autopsy.

It will be noticed that there was no difference in the results in the exposure in the clean, disinfected pen and the exposure in the original, contaminated pen; in other words, the contact of the susceptible pigs with the sick pigs was the essential factor in the conveyance of the disease, and the additional pen infection in the one case had no apparent effect on the development of the disease in the exposed pigs.

In this experiment the disease was transmitted by contact as late as the tenth day after infection, or the seventh day after the appearance of the first visible symptoms.

EXPERIMENT VII.—Pig 932 was injected with 5 c. c. of virus blood on October 25, showed first visible sickness on October 30, and developed the usual cholera symptoms. This pig was transferred to a clean, disinfected pen on November 11, 17 days after inoculation and 12 days after the first appearance of visible symptoms, and two susceptible pigs, No. 1140 and 1141, were placed in the same pen. At the time of transfer, pig 932 was off feed and showed a fever temperature, conjunctivitis, weakness, and diarrhea.

The two exposed pigs, No. 1140 and 1141, contracted cholera from contact with the sick pig and showed first visible symptoms on November 20, 9 days after exposure. Pig 1140 was killed when in a moribund condition, on November 25, showing extensive hemorrhagic lesions at autopsy. Pig 1141 died November 25 and exhibited extensive hemorrhagic lesions. Hog 932, which furnished the exposure, was found dead on November 16 and showed characteristic hemorrhagic lesions of cholera.

In this experiment the disease was transmitted by contact as late as the seventeenth day after inoculation, or the twelfth day after the appearance of first visible symptoms.

EXPERIMENT VIII.—Pig 794 was injected with 5 c. c. of virus blood on June 30. The animal was first visibly sick on July 7 and developed characteristic cholera symptoms. On July 21, 21 days after inoculation and 14 days after the appearance of visible symptoms, this pig was first scrubbed with soap and water, next with compound cresol solution, again with soap and water, and was transferred to a clean, disinfected pen. A susceptible pig, No. 811, was placed in the same pen. At the time of transfer, pig 794 showed a temperature of 103.8° and was recorded as off feed and as showing conjunctivitis, diarrhea, and red skin.

The exposed pig, No. 811, contracted hog cholera from contact with the sick pig, showing the first visible symptoms on July 25, four days after exposure; this pig died on July 30, and the autopsy revealed hemorrhagic lesions. Pig 794, which furnished the exposure, died on August 4 and showed hemorrhagic lesions and ulceration of cecum and colon.

In this experiment the disease was transmitted by contact as late as the twenty-first day after inoculation, or the fourteenth day after the appearance of first visible symptoms.

EXPERIMENT IX.—Pig 904 contracted hog cholera by association with sick pigs and developed characteristic cholera symptoms, including weakness, loss of appetite, conjunctivitis, and diarrhea. This pig was transferred to a clean, disinfected pen on October 28, 21 days after the appear-

ance of first visible symptoms, and two susceptible pigs, No. 1093 and 1094, were placed in same pen. At the time of transfer pig 904 was recorded as being off-feed, in poor condition, and as showing diarrhea.

The two exposed pigs, No. 1093 and 1094, contracted cholera from contact with the sick pig and showed the first visible symptoms on November 3. Both of these pigs died on November 10. Pig 1094 showed hemorrhagic lesions at autopsy and No. 1093 showed hemorrhagic lesions with ulceration of cecum. Pig 904, which furnished the exposure, died on October 30, showing hemorrhagic lesions with extensive ulceration of the cecum and colon.

In this experiment the disease was transmitted by contact as late as the twenty-first day after the appearance of first visible symptoms.

In the four experiments which have just been described, susceptible pigs were exposed to sick pigs on the seventh, twelfth, fourteenth, and twenty-first days after the appearance of first visible symptoms of sickness. All of the exposed pigs contracted hog cholera as a result of the exposure.

The preceding experiments, No. V to IX, inclusive, show that hog cholera is contagious at all stages, even including the stage of incubation. They indicate also that an infected hog remains a source of danger at least until the time of complete recovery.

RECOVERED PIGS AS CARRIERS OF HOG CHOLERA

Suspicion has long rested on the recovered pig as a possible carrier of hog cholera, but there seems to be little, if any, experimental evidence on this point. This lack of experimental evidence is no doubt largely due to the difficulty of obtaining hogs which have had genuine attacks of cholera and have subsequently made a complete recovery.

In the course of the experiments which were carried out during the summer and fall of 1916, four pigs were secured which had suffered from acute, typical hog cholera, and which had made apparently good recoveries. The following experiments were carried out with these animals.

EXPERIMENT X.—Pig 893 was injected with virus on September 13, 1916, and within the usual time developed an acute case of cholera, but made an apparently good recovery. The clinical record of this pig is given in full in Table IX.

TABLE IX.—*Record of recovered pig 893*

Date.	Temperature.	Symptoms.
1916.	°F.	
September 13	101.7	Normal.
September 14	102.8	Do.
September 15	102.8	Do.
September 16	105.6	Slow.
September 17	Not taken.	Off-feed.
September 18	105.0	Do.
September 19	104.0	Do.
September 20	105.4	Off-feed, weakness, conjunctivitis, and purple ears.
September 21	104.6	Do.
September 22	104.1	Do.
September 23	105.0	Do.
September 24	Not taken.	Do.
September 25	102.3	Do.
September 26	102.5	Eating a little.
September 27	102.3	Eating a little; improvement.
September 28	103.0	Do.
September 29	102.3	Do.
September 30	102.3	Do
October 1	Not taken.	Eating more; improvement.
October 2	101.9	Do.
October 3	101.0	Normal.
October 4	102.0	Do.
October 5	101.6	Do.

On October 6, when this hog had entirely recovered, it was thoroughly scrubbed, first with soap and water, and next with compound cresol solution, and again with soap and water. The pig was then transferred to a clean, disinfected pen, and a susceptible pig, No. 1029, was placed in the same pen. The two pigs were in close association from October 6 to 26, a period of 20 days, during which time the susceptible pig remained perfectly well. Pig 1029 was injected with virus on October 27 to test its susceptibility and died on November 4, eight days later, with well-marked hemorrhagic lesions.

In order to determine whether pig 893 harbored the virus of hog cholera in its blood, this animal was bled from the tail on October 6 and 5 c. c. of the defibrinated blood was injected into pig 1031. Pig 1031 was kept under careful observation from October 6 to 27, a period of three weeks, and remained perfectly normal. Pig 1031 was injected with 5 c. c. of virus on October 27 to test its susceptibility. It contracted cholera and was killed for virus eight days later, the autopsy revealing well-marked hemorrhagic lesions and beginning ulceration of the cecum.

EXPERIMENT XI.—Pig 951 was injected with virus on August 29, was off-feed on the fifth day, and developed a marked temperature reaction and conjunctivitis, but made a good recovery. The clinical record of this pig following the virus injection is given in Table X.

TABLE X.—*Record of recovered pig 951*

Date.	Temperature.	Symptoms.
1916.	°F.	
August 30	103.6	Normal.
August 31	104.4	Do.
September 1	104.5	Do.
September 2	104.4	Do.
September 3	.....	Off-feed.
September 4	106.1	Do.
September 5	106.4	Off-feed; conjunctivitis.
September 6	105.6	Do.
September 7	106.2	Do.
September 8	101.7	Do.
September 9	103.8	Do.
September 10	.....	Do.
September 11	103.4	Eating a little.
September 12	103.0	Do.
September 13	102.8	Do.
September 14	103.5	Do.
September 15	103.2	Do.
September 16	102.1	Eating better.
September 17	.....	Do.
September 18	103.5	Do.
September 19	102.0	Normal.
September 20	102.6	Do.
September 21	102.0	Do.

On September 21 pig 951 was thoroughly washed, first with soap and water, then with compound cresol solution, and again with soap and water, and was then transferred to a clean, disinfected pen. A susceptible pig, No. 1011, was then placed in the pen with pig 951. The two pigs were kept in the pen together from September 21 to October 19, a period of four weeks, and during this time the pen check or control remained perfectly normal. On October 19 the control pig, No. 1011, was removed and injected with virus blood to test its susceptibility; the animal sickened as a result of the injection, was off its feed for a week, and showed a marked temperature reaction, but recovered.

In order to determine whether the virus was present in the blood of pig 951, this animal was bled from the tail on October 6 and 5 c. c. of the defibrinated blood were used for the injection of pig 1030. This animal was kept under observation from October 6 to October 27, a period of three weeks, and remained perfectly well. On October 27 it was injected with 5 c. c. of virus blood to test its susceptibility and was killed for virus eight days later, the autopsy showing characteristic hemorrhagic lesions and ulceration of cecum.

EXPERIMENT XII.—Pig 1046 was injected with 5 c. c. of urine from a cholera-sick pig, No. 1032x, on October 13, and developed a typical, acute case of hog cholera, but made a good recovery. This pig was one of those used in the experiment to determine the infectiousness of the blood, excreta, and secretions of cholera-infected pigs. Its record is

included in Table II. The clinical record of this pig is given in full in Table XI.

TABLE XI.—*Record of recovered pig 1046*

Date.	Temperature.	Symptoms.
1916.	°F.	
October 14	102.4	Normal.
October 15	.....	Do.
October 16	102.6	Do.
October 17	102.6	Do.
October 18	102.6	Do.
October 19	103.0	Do.
October 20	102.6	Do.
October 21	101.6	Do.
October 22	.....	Do.
October 23	104.6	Do.
October 24	106.1	Slow.
October 25	105.6	Off-feed.
October 26	104.8	Ate some feed.
October 27	104.7	Off-feed.
October 28	104.0	Do.
October 29	.....	Off-feed; red ears.
October 30	104.5	Do.
October 31	105.3	Off-feed; red ears, and diarrhea.
November 1	105.0	Do.
November 2	103.8	Do.
November 3	105.0	Do.
November 4	104.4	Do.
November 5	.....	Do.
November 6	102.8	Ate some feed.
November 7	102.2	Do.
November 8	105.0	Do.
November 9	102.8	Do.
November 10	103.8	Do.
November 11	104.0	Do.
November 12	.....	Ate feed; general improvement.
November 13	103.6	Do.
November 14	103.2	Do.
November 15	104.6	Do.
November 16	103.2	Do.
November 17	103.0	Normal.
November 18	103.0	Do.
November 19	.....	Do.
November 20	102.6	Do.
November 21	102.0	Do.
November 22	103.0	Do.
November 23	.....	Do.
November 24	.....	Do.
November 25	102.6	Do.

On November 24, two susceptible pigs, Nos. 1180 and 1181, were placed in the pen with pig 1046. In this instance pig 1046 was not scrubbed; nor was the pen disinfected. The three pigs were in close association from November 24 to December 15, a period of 21 days, during which time they remained well. All three pigs were injected with virus on December 15. Pig 1046 remained well. Pigs 1080 and 1081 became sick on December 20, showing loss of appetite, and some elevation of temperature, but recovered and were reported normal on December 29.

This experiment is not quite as satisfactory as the three others, as the two exposed pigs did not die as a result of the virus injection which was given them in order to test their susceptibility. They undoubtedly possessed some degree of natural immunity, but the fact that they both sickened after the virus injection serves to establish their susceptibility.

EXPERIMENT XIII.—Pig 1070 was injected with 5 c. c. of a normal salt dilution of nasal secretion from pig 1032x on October 16, 1916, and developed a typical, acute case of hog cholera, but made a good recovery. This pig was one of those used in the experiment to determine the infectiousness of the blood, excreta, and secretions of cholera hogs, the record of which is included in Table II.

The clinical record is given in full in Table XII.

TABLE XII.—*Record of recovered pig 1070.*

Date.	Temperature.	Symptoms.
1916.	°F.	
October 17	103.6	Normal.
October 18	103.7	Do.
October 19	103.1	Do.
October 20	103.8	Do.
October 21	106.5	Off feed.
October 22	.....	Do.
October 23	107.0	Do.
October 24	105.5	Do.
October 25	106.7	Do.
October 26	106.5	Off feed; purple ears.
October 27	106.1	Do.
October 28	105.9	Off feed; conjunctivitis; weakness.
October 29	.....	Do.
October 30	104.8	Do.
October 31	103.0	Do.
November 1	103.8	Do.
November 2	102.7	Do.
November 3	103.6	Ate some feed.
November 4	103.9	Do.
November 5	.....	Do.
November 6	105.0	Off feed; diarrhea.
November 7	100.6	Do.
November 8	102.9	Do.
November 9	103.8	Do.
November 10	102.9	Do.
November 11	101.0	Ate some feed; diarrhea.
November 12	101.0	Do.
November 13	102.1	Do.
November 14	101.4	Do.
November 15	101.6	Do.
November 16	102.0	Do.
November 17	.....	Do.
November 18	101.0	Do.
November 19	101.0	Do.
November 20	102.3	Do.
November 21	102.8	Do.
November 22	102.8	Do.
November 23	102.8	Do.
November 24	104.4	Do.
November 25	103.0	Ate feed.

TABLE XII—*Record of reovered pig 1070*—Continued.

Date.	Temperature.	Symptoms.
1916.	°F.	
November 26	103.0	Ate feed.
November 27	103.4	Normal.
November 28	102.8	Do.
November 29	102.8	Do.
November 30	.....	Do.
December 1	102.0	Do.
December 2	101.9	Do.
December 3	.....	Do.
December 4	.....	Do.
December 5	103.1	Do.

From the above record it will be seen that hog 1070 had a typical and well-marked case of hog cholera, the temperature going as high as 107°. On December 4, eight days after the animal was recorded as normal, two suceptible pigs, Nos. 1196 and 1197, were placed in the pen with hog 1070. In this experiment the pen was not disinfected; nor was the recovered pig washed and disinfected as in the first two experiments. The three pigs were kept together from December 5 to 27, a period of 22 days, during which time they remained well.

All three pigs were injected with virus on December 27 to test their immunity. Hog 1070 remained well. Pigs 1196 and 1197 became sick on January 1, 1917, developed the usual symptoms of hog cholera, and were killed for virus on January 9, each showing well-marked hemorrhagic lesions of hog cholera at autopsy.

In the four experiments which have just been described, recovered pigs which had suffered from typical attacks of hog cholera were tested by exposure with susceptible pigs and also by the withdrawal of blood and the inoculation of susceptible pigs to determine whether they harbored the virus within their bodies and could act as carriers of the disease. In all four experiments the results were entirely negative.

These experiments do not, of course, show that recovered pigs may not at times be carriers of hog cholera; yet, on the contrary, they do prove that all recovered pigs are not carriers. When these results are considered with those of experiments I to VI, we may conclude, that although hogs which have been infected with cholera are dangerous so long as they show any symptoms of cholera, they may lose the power to convey the disease to others, once they have made a good recovery.

PIGEONS AS CARRIERS OF HOG CHOLERA

The belief that birds play an important part in the dissemination of hog cholera is based largely upon practical observations, such as an outbreak of cholera on a farm frequented by pigeons that are known to

fly to and from an infected farm. This belief is founded also upon the knowledge that the virus of cholera exists in the carcasses of dead hogs and on premises occupied by herds that are infected with the disease. It is reasonable to assume that any agency likely to carry bits of tissue from carcasses of hogs that have died of cholera, as for example, buzzards, or any agency likely to carry particles of dirt from an infected hog lot, as, for example pigeons, may serve to disseminate hog cholera. However, although there is ample ground for suspecting that certain birds do at times carry the disease from one farm to another, absolute and convincing evidence upon this point is lacking. The following experiments were carried out with the object of securing definite experimental data relative to the likelihood of the conveyance of hog cholera by pigeons.

Two pens, 5 feet square, were placed facing each other, 10 feet apart, and the space between was inclosed with wire netting. Small hinged doors were cut in the sides of the pens, so that the pigs could be fed from the outside. The upper portion of the front of each pen was left open. The pens bore the numbers 19 and 22 (fig. 1).

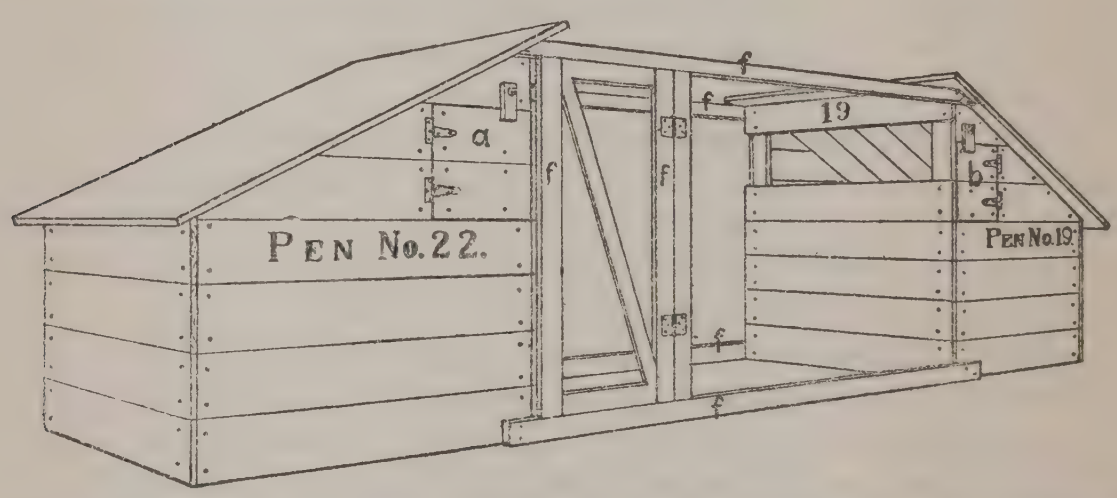

FIG. 1.—Diagram showing the arrangement of pens for pigeon experiments: *a*, *b*, Doors for feeding and watering pigs; *f*, *f*, framework for holding wire netting.

EXPERIMENT XIV.—Two pigs, No. 2283 and 2284, were injected with hog-cholera virus and placed in pen 19 on September 18. Two uninoculated, susceptible pigs, No. 2285 and 2286, were placed in pen 22 on the same date. Six pigeons were placed within the wire inclosure, the birds having free access to both pens through the open fronts. No food was placed in the space between the pens and the birds were forced to go into the pens with the pigs to secure their food.

The two injected pigs in pen 19 developed characteristic cholera symptoms and died on September 26, showing extensive hemorrhagic lesions and intestinal ulceration at autopsy. The pigeons were frequently in the pen with the sick pigs and even sat on the bodies of the sick pigs when these were in a moribund condition. They were also frequently in the pen with the exposed pigs.

Two more pigs, No. 2317 and 2318, were injected with virus and placed in pen 19 on September 30, to replace the two pigs which had died there and to keep up the infection in this pen. Both of these pigs developed cholera, No. 2317 dying on October 8 and No. 2318 on October 18. Both animals showed extensive hemorrahgic lesions at autopsy.

The two uninoculated pigs, No. 2285 and 2286, in clean pen 22 remained perfectly well from September 18 to October 18, a period of 30 days, in spite of the fact that during this time they were daily subjected to possible infection from virus carried on the feet of the pigeons, which divided their time between the pens, flying freely from side to side. In order to test the susceptibility of pigs 2285 and 2286, and at the same time prove that the virus of hog cholera was present in the litter of pen 19, these pigs, without being treated in any way, were transferred from clean pen 22 to infected pen 19 on October 18. Both pigs picked up the infection from the pen and developed typical hog cholera, pig 2286 dying on November 6 and pig 2285 on November 17. At autopsy both pigs revealed extensive hemorrhagic lesions and intestinal ulceration. It was thus shown that the two pigs which were exposed to the pigeons for 30 days were susceptible pigs and that the virus of the hog cholera was present in the litter of pen 19, but was not carried over by the pigeons.

Experiment XV.—Two uninoculated, susceptible pigs, No. 2457 and 2458, were placed in clean pen 22, on October 20, for exposure to the pigeons. During the period from October 20 to November 17, pen 19 was occupied by infected pigs (see Experiment XIV). Upon the death of the last of these, on November 17, two more uninoculated, susceptible pigs, No. 2766 and 2767, were placed in infected pen 19. These two pigs, like their immediate predecessors, picked up the infection from the pen, and developed typical hog cholera. Both died on November 27, showing extensive hemorrhagic lesions and intestinal ulceration.

On November 29 the pigeons, which had alighted and fed in both pens 19 and 22 continually since October 20, were shut off from pen 22. They remained excluded until December 15, in order to give time for any infection that had been carried by them to develop in the two susceptible pigs, No. 2457 and 2458. These two pigs continued perfectly well throughout the entire period, although they had been exposed to possible infection by the pigeons for 40 days, from October 20 to November 29.

On December 15, pigs 2457 and 2458, having failed to contract disease from exposure to the pigeons, were transferred from pen 22 to pen 19, and each injected with 5 c. c. of hog-cholera virus. This was done in order to test the susceptibility of these pigs and to furnish fresh infection to pen 19. Both pigs developed hog cholera following the virus injection and both died on January 7, 1916, exhibiting extensive hemorrhagic lesions and intestinal ulceration at autopsy.

EXPERIMENT XVI.—Two susceptible pigs, No. 2939 and 2940, were placed in pen 22 on December 16, and the pigeons were allowed free access to this pen, as well as to pen 19, which then contained infected pigs 2457 and 2458 (see Experiment XV). The last-named pigs died on January 7, and were removed from pen 19, which remained unoccupied until January 17, when susceptible pigs No. 3480 and 3481 were each injected with 5 c. c. of blood from a sick pig and placed in pen 19. Both of these pigs contracted cholera from the inoculation; one died on January 31, and the other on February 1, and both exhibited extensive lesions of hog cholera at autopsy.

Pigs 2939 and 2940 were kept in pen 22 from December 16 to February 5. For 37 days of this period infected pigs were kept in pen 19, while during the entire period of 51 days the pigeons had free access to both pens. On February 5, of pigs 2939 and 2940, which had remained well, were each injected with 5 c. c. of virus blood to test their susceptibility. Both developed hog cholera as a result of the injections.

Pig 2939 died on February 14, showing extensive hemorrhagic lesions at autopsy, and hog 2940 died on February 27, showing extensive hemorrhagic lesions and intestinal ulceration.

The results of the three experiments with pigeons as carriers of hog cholera are epitomized in Table XIII.

TABLE XIII.—*Results of tests to determine whether pigeons are disseminators of hog cholera*

Pig No.	Date placed in pen 22.	Purpose.	Length of time exposed to pigeons.	Remarks.
	1915.			
2283	.....	.....	.....	
2284	.....	.....	.....	
2317	.....	.....	.....	
2318	.....	.....	.....	
2285	Sept. 18	Exposure to pigeons.....	30 days..	Remained well while in pen 22.
2286	Sept. 18	.....do.....	30 days..	Do.
2766	.....	.....	.....	
2767	.....	.....	.....	
2457	Oct. 20	Exposure to pigeons.....	40 days..	Remained well while in pen 22.
2458	Oct. 20	.....do.....	40 days..	Do.
2939	Dec. 16	.....do.....	37 days..	Remained well while in pen 22. Injected with 5 c. c. of virus on Feb. 5, 1916.
2940	Dec. 16	.....do.....	37 days..	Do.
3480	.....	.....	.....	
3481	.....	.....	.....	

TABLE XIII.—*Results of tests to determine whether pigeons are disseminators of hog cholera*—Continued

Pig No.	Date placed in pen 19.	Purpose.	Died.	Post-mortem lesions.
	1915.		1915.	
2283	Sept. 18	Injected with 5 c. c. of virus to furnish pen infection.	Sept. 26	Extensive hemorrhages and intestinal ulceration.
2284	Sept. 18	.....do.....	Sept. 26	Do.
2317	Sept. 30	.....do.....	Oct. 8	Extensive hemorrhages.
2318	Sept. 30	.....do.....	Oct. 18	Do.
2285	Oct. 18	To test susceptibility and furnish pen infection.	Nov. 17	Extensive hemorrhages and intestinal ulceration.
2286	Oct. 18	.....do.....	Nov. 6	Do.
2766	Nov. 17	To continue pen infection.....	Nov. 27	Do.
2767	Nov. 17	.....do.....	Nov. 27	Do.
			1916.	
2457	Dec. 15[a]	Injected with 5 c. c. of virus to test susceptibility and furnish pen infection.	Jan. 7	Do.
2458	Dec. 15[a]	.....do.....	Jan. 7	Do.
2939	.....	.....	Feb. 14	Extensive hemorrhages.
2940	.....	.....	Feb. 27	Extensive hemorrhages and intestinal ulceration.
	1916.			
3480	Jan. 17	Injected with 5 c. c. of virus to furnish pen infection.	Feb. 1	Extensive hemorrhages.
3481	Jan. 17	.....do.....	Jan. 31	Do.

[a] Pigeons were shut out of pen 22 from November 29 to December 15 in order to give time for any infection carried by them to develop in the exposed pigs.

In these experiments three lots of pigs, each lot consisting of two pigs, were exposed to possible infection from virus carried by pigeons, at a distance of 10 feet from a heavily infected pen. In the experiments the periods of exposure were 30, 37, and 40 days, respectively, with negative results in each case. In these tests the exposure of susceptible pigs to possible infection from virus carried by pigeons was severe and long continued. The pen which contained the sick pigs was not cleaned during the course of the experiment and became very foul from the excreta of the sick pigs; the pigeons were constantly passing from the heavily infected pen to the pen containing the well pigs; and the distance between the two pens was only 10 feet. Every opportunity was afforded, therefore, for prolonged periods of time, for the pigeons to carry the infection a very short distance.

It goes without saying that these experiments do not prove that it is impossible for pigeons to convey hog cholera. They do indicate, however, that the disease is probably not often carried in that way.

EXPERIMENTS TO DETERMINE WHETHER RATS MAY HARBOR THE VIRUS OF HOG CHOLERA

In considering the various agencies which might be concerned in the spread of hog cholera, the possibility suggested itself that rats which had fed on the carcasses of cholera pigs might play a part in the spread of the disease, and the following preliminary experiments were carried out to test this point:

EXPERIMENT XVII.—Two gray rats which were caught on the Station premises were fed daily for five days with meat from the carcasses of

cholera-infected pigs. The two rats were then killed; the bodies were chopped up in their entirety and, mixed with bran mash, were fed to two susceptible pigs, No. 1118 and 1119.

The two pigs were kept under observation from November 4 to 24, a period of 20 days, and remained well. They were exposed to hog cholera by virus injection on November 24, showed the first symptoms of sickness on November 29, developed the usual cholera symptoms, and were killed for virus on December 1. Both pigs showed slight but characteristic lesions at autopsy.

EXPERIMENT XVIII.—Two gray rats which were caught on the Station premises were fed daily from October 31 to November 20, a period of 21 days, with the meat of cholera-infected pigs. The two rats were killed on November 21. They were then chopped up, mixed with bran mash, and fed to two susceptible pigs, No. 1174 and 1175. The pigs were under observation from November 21 to December 8, a period of 17 days, and remained well. The pigs were exposed to hog cholera by virus injection on December 8, showed the first visible symptoms on December 11, developed the usual cholera symptoms, and were killed for virus on December 15. Each pig showed hemorrhagic lesions of hog cholera at autopsy.

In these experiments rats which had been fed on the meat of cholera pigs for 5 days and 21 days, respectively, were killed and the entire carcasses fed to susceptible pigs without producing sickness.

GENERAL SUMMARY AND CONCLUSIONS

Although the data obtained from these experiments are not sufficient to warrant sweeping conclusions, the results are nevertheless quite suggestive, and they serve to bring out some interesting points which may be summarized as follows:

(1) The eye and nose secretions, the blood, the urine, and the feces of cholera-infected pigs were tested on the first, second, third, fifth, seventh, and ninth days after infection. When injected, the eye and nose secretions and fecal suspensions, were found to be infectious on the third day; the urine was quite regularly infectious by the fourth or fifth day and the blood was infectious as early as the first day. When fed and when scattered in pens, the freshly collected secretions and excreta were noninfectious as a rule. Secretions and excreta which were held at room temperature (60° to 85° F.) for 24 hours remained infectious when injected. When the secretions and excreta were held at the same temperature for 48 hours the urine and feces remained infectious, but the eye and nose secretions were no longer so. It might appear, therefore, that outside the animal body the virus in the eye and nose secretions succumbs more quickly than the virus

in the urine and feces, but such a conclusion is not justified by these experiments, as the virus from the eye and nose was allowed to dry on swabs. This point requires further study with the virus from the different sources held under identical conditions. Finally, it should be noted that the eye and nose secretions may be infectious before there is any visible discharge from the eyes or nose.

(2) Susceptible pigs were exposed by association with cholera-infected pigs for 48-hour periods on the first, third, fifth, seventh, ninth, and eleventh days after infection. With the exception of those exposed on the first and second days—that is, during the first 48-hour interval—all of the exposed pigs contracted hog cholera. Other pigs which were exposed to cholera-infected pigs at 17 and 21 days contracted hog cholera. Cholera-infected pigs therefore may transmit the disease by contact at practically all stages of the disease, even in the period of incubation, before the appearance of visible symptoms and before the animal can be recognized as sick.

(3) Susceptible pigs were exposed by being placed in pens with pigs which had suffered from typical attacks of hog cholera but had recovered. Other susceptible pigs were inoculated with blood drawn from the recovered pigs. Four recovered pigs were tested in this way to determine whether they harbored the virus of cholera within their bodies and might act as carriers of the disease. None of the pigs exposed to the recovered pigs, either by association or by blood injection, developed hog cholera. The exposed pigs were later proved to be susceptible by virus injection.

(4) Susceptible pigs were exposed for long periods of time to pigeons, which passed daily from a heavily infected pen only 10 feet away and which contained sick and dying pigs, to a pen containing susceptible pigs. The exposure in these experiments was severe, as the pigeons were afforded every opportunity to carry the infection over a very short distance. Notwithstanding this, none of the exposed pigs developed cholera. All of the exposed pigs were later proved to be susceptible either by virus injection, by association directly with sick pigs, or by exposure in an infected pen. These experiments extended through the fall and well into the winter. While the assumption would hardly be warranted that pigeons never convey hog cholera, it does not seem likely that they are often concerned in the spread of this disease.

(5) Rats were fed on the meat of cholera hogs for periods of 5 and 21 days. The rats were then killed, their entire bodies chopped up, mixed with bran mash, and the mixture was fed to susceptible pigs. None of the pigs thus fed contracted cholera. The pigs were proved to be susceptible by subsequent virus injection.

EFFECT OF TEMPERATURE AND OTHER METEOROLOGICAL FACTORS ON THE GROWTH OF SORGHUMS

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HISTORICAL REVIEW

The botanical group *Andropogon sorghum* includes an extensive and widely varying list of cultivated crop plants which are primarily subtropical in their climatic requirements, although many varieties are now being grown well north in the temperate regions. The effect of temperature and other meteorological factors on the sorghums has not been discussed very extensively in agricultural literature.

Sachs (*5, p. 365*)[1] determined the minimum, optimum, and maximum temperatures for wheat, barley, pumpkin, beans, and corn as follows:

Plant.	Minimum.	Optimum.	Maximum.
	°*F.*	°*F.*	°*F.*
Wheat	41	83.7	108.5
Barley	41	83.7	99.9
Pumpkin	56.7	92.7	115.2
Beans	49.1	92.7	115.2
Corn	49.1	92.7	115.2

The above figures, however, are for seedlings (germinating seeds), and while the results are indicative, it is probably true that the more fully developed plants would continue to grow under wider extremes.

The work of other investigators, Mayer, Heinrich, etc., indicate that the optimum temperatures for the growth of the nasturtium (*Tropaeolum majus*), the water violet (*Hottonia palustris*), and white mustard (*Sinapis alba*) also lie between 88° and 95° F. Bose (*3, p. 445*) found the optimum temperature for the Crinum lily, the peduncle of the crocus, and and the hypocotyl of the balsam to be 95° F.

It would seem from these tests that the optimum temperature for a considerable number of plants is between 83° and 95° F. From the

[1] Reference is made by number (italic) to "Literature cited," p. 147.

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writers' knowledge of sorghum they have reason to believe that its temperature requirements resemble those of beans (Fabaceae) and corn (*Zea mays*) more closely than they do those of wheat (*Triticum* spp.) and barley (*Hordeum* spp.) The optimum temperature for the growth of sorghum is quite likely about 92° or 93°. Above this optimum, growth is retarded by further increases in temperature until the maximum is reached, when growth ceases entirely, and continued exposure to the maximum temperature will cause death. Just how the high temperatures (those above the optimum) effect this retardation of the growth is not well understood. Vines (*8, p. 283-284*) states that many physiologists believe the fatal effect of high temperatures is due to the coagulation of the coagulable proteids of the cell, but this has not been proved. He goes on to say:

> It is doubtless upon the living protoplasm of the cell that the temperature acts; the effect first manifests itself by a diminution of the metabolic activity of the protoplasm, and ultimately effects its disorganization.

Kreusler found that assimilation in species of Ricinus and Rubus begins to decrease at 86° and at 113° F. is stopped almost entirely.

Balls (*2*), who worked on the "sore shin" fungus of cotton, attributed this decrease of assimilative power and growth in the presence of high temperatures to the accumulation of katabolic products in the cell and not to any inability of the protoplasm to function at temperatures of 98° to 100° F., which he determined as the practical stopping point of growth in the "sore-shin" fungus.

Ewart's (*4, p. 385–387*) work seems to confirm Balls's theory. He found that plants of corn, beans, pumpkins, etc., subjected for three days to temperatures of 98° to 100° F., if well supplied with oxygen and moisture, showed no inhibitory after effect, but if the supply of air were limited a retardation of assimilation was induced which persisted for sometime after the temperature had been reduced to a point most favorable for the plant's growth. Thus, it would appear that it may be a failure properly to dispose of the products of katabolism which interferes with continued assimilation and growth at superoptimal temperatures.

Carefully controlled tests would have to be made, of course, in order to determine whether sorghum behaves as these other plants have done under similar temperatures; but observations in the field at Bard, Cal., indicate that there is a slowing up of growth in nearly all field crops during the hottest part of the summer, when air temperatures in the daytime are often consistently above 100° F.

The manner in which low temperatures act upon the plant to decrease its rate of growth has been studied less than the effect of high temperatures. The minimum temperature for growth has been determined for a number of plants, and several investigators have studied in detail

the effect of freezing plants; very few of these, however, have considered the effect of suboptimal temperatures on the retardation of growth. It has been observed that the processes of metabolism become less pronounced at low temperatures. This slowing up of the growth is due perhaps to a decrease in enzymic action, as well as to the failure of the protoplasm to function properly at such temperatures.

Although light is necessary for the normal development of chlorophyllous plants, but little is known regarding the effect of different intensities of light on their growth. Sachs (*6*) found that the rate of growth in the seedlings of maize increases during the night and decreases during the day, the period of greatest growth being early in the morning just as it is becoming light. Smith (*7*), in testing Blackman's theory of limiting factors, made an extensive series of growth measurements on the new culms or growing shoots of bamboo. In all but one instance these measurements showed a more rapid growth at night than in the daytime. Smith concluded there were two factors which controlled the growth: (1) temperature of the culm, and (2) supply of water to the culm. The second factor, being intimately connected with the amount of water drawn off by the transpiration of adult culms on the same rhizome, was influenced in its turn by two factors: First, the humidity of the atmosphere, and second, the intensity of the light. At night the greater humidity of the atmosphere and the lessened transpiration due to the absence of light both contributed to the certainty of an adequate supply of water for the growing shoot.

The measurements of Sachs and of Smith were made on growth produced from reserve food stored in the plant itself, and the results can not be applied to the behavior of general farm crops like sorghum, for it is known that in green plants the continued absence of light results in etiolation and eventually in a cessation of growth. A certain amount of light is ordinarily required in the formation and functioning of chlorophyll. Without sunlight the green plant is powerless to form new organic compounds, and its growth in darkness comes about only through the transformation of organic compounds already at its disposal.

The observations of Linsser, Marie-Davy, and Angot, as noted by Abbe (*1, p. 211–290*), show that there exists a decided difference in the quantity of heat necessary for the development of the same species of plants in different latitudes, and Marie-Davy asserts that the determining influence is the quantity of light which the plants receive. Abbe (*1, p. 79*) also points to some work of Hellriegel with barley, in which it was found that plants in the open air made a 50 per cent larger yield than those grown inside a greenhouse in the direct sunshine, and fully three times as much as plants grown in diffuse light under glass.

OUTLINE OF EXPERIMENTS AND DESCRIPTION OF WEATHER CONDITIONS

In the preceding discussion literature has been cited only for the purpose of an introduction to the presentation of the data obtained in the following experiments. No attempt has been made to make the review of the literature complete or even extensive. It is believed that the limited data obtained in the field tests hereafter described will be of value not only in establishing the climatic limitations of the sorghums but also in indicating the principles which underlie the results of numerous "Date of planting" experiments with this crop.

It has been noticed that the sorghums behave very peculiarly in localities which have continuously low temperatures. In 1915 an attempt was made to obtain data on this effect of low temperatures by growing certain selected varieties of sorghum under widely varying climatic conditions. Plantings were made at Puyallup, Wash.; Chico, Berkeley, Bard, and Pasadena, Cal.; and Chillicothe, Tex. It was thought that these points represented the extremes of humidity and heat, as well as the more intermediate conditions. At Puyallup the temperatures and the percentage of sunshine are low through the summer, and the nights are cool. At Berkeley there is high humidity with moderate temperatures, while at Chico and Bard the temperatures are high, the humidity low, and the sunshine abundant. At Pasadena and Chillicothe somewhat intermediate climatic conditions prevail.

Very complete notes were made regarding the growth of these sorghums at Berkeley, but the plots were not irrigated; and as there was practically no rain from May until September, the lack of soil moisture may have exerted as much influence on their growth as did the weather factors. It has seemed best, therefore, to substitute for the Berkeley results of 1915 those obtained at Chula Vista in 1916. Summer conditions at Chico and Bard, Cal., are so similar and the results so nearly alike that only the results at Bard are given. In like manner Chillicothe was chosen to represent intermediate climatic conditions.

Table I gives in considerable detail the chief features of the weather at Chillicothe, Bard, and Puyallup in 1915 and Chula Vista in 1916, showing the maximum, minimum, mean, and normal temperatures, the mean relative humidity, the inches of rainfall, and the percentage of actual to possible sunshine for each month.

For Chillicothe only the rainfall and mean monthly temperature records were obtained directly at that point. The relative humidity and percentage of sunshine are those recorded by the United States Weather Bureau at Abilene, Tex., and the remainder of the data was taken from the Weather Bureau records for Quanah, Tex., 13 miles west of Chillicothe. It is probable that the relative humidity at Abilene, 130 miles south of Chillicothe, is a trifle too low and the percentage of sunshine may

be 3 or 4 per cent too high, but the figures represent very closely condiditions at Chillicothe.

The data for Bard were taken from the Weather Bureau records for Yuma, Ariz., which is only 7 miles distant and is practically the same elevation as Bard.

The data as given for Chula Vista, Cal., are taken from the Weather Bureau records at San Diego, Cal., which is 12 miles from Chula Vista and similarly located in respect to its proximity to the ocean.

There were no complete weather records for Puyallup, Wash., at any point nearer than Tacoma, Wash., 8 miles distant, but since these two places are very similarly located, it is believed that the data from Tacoma represent conditions at Puyallup closely enough for the purposes of this study.

TABLE I.—*Weather conditions during 1915 and 1916*

CHILLICOTHE, TEX., 1915 [a]

Month.	Temperature (° F.).						Mean relative humidity.	Rainfall.	Percentage of actual to possible sunshine.
	Maximum.		Minimum.		Mean for month.	Normal for month.			
	Mean.	Absolute.	Mean.	Absolute.					
							Per cent.	*Inches.*	
January	51.7	68	32.0	18	38	40.5	65	0.34	70
February	60.3	74	40.8	31	46	40.2	56	1.88	70
March	53.5	86	39.2	29	41	52.1	67	1.22	50
April	74.1	89	56.8	42	63	63.5	69	5.13	60
May	79.1	93	57.4	45	68	70.5	64	2.15	75
June	89.2	103	61.7	54	78	79.6	62	6.71	80
July	95.7	109	70.1	58	81	83.3	56	4.07	75
August	91.0	107	65.6	51	76	82.5	63	3.73	75
September	89.6	106	66.6	51	75	76.6	.....	3.83	70
October	80.1	90	54.2	41	63	63.4	69	5.07	85
November	72.6	95	42.8	24	55	51.7	52	.15	85
December	62.7	82	32.3	19	46	40.2	58.5	.53	65
Yearly	75.0	109	51.6	18	60.8	62.0	61.9	34.81	71.7

BARD, CAL., 1915 [b]

January	64.9	74	42.6	34	53.7	54.7	52.5	2.56	72
February	68.8	73	44.7	37	56.8	59.2	57.0	.72	81
March	79.2	91	50.0	36	64.6	64.7	44.5	T.	90
April	83.7	97	54.4	47	69.0	70.1	46.0	.08	89
May	88.9	104	56.3	39	72.6	76.8	43.0	T.	93
June	104.3	112	66.5	59	85.4	84.7	40.0	0.00	98
July	104.8	110	75.2	63	90.0	90.9	45.0	.34	93
August	107.7	116	74.4	67	91.0	91.9	45.0	.41	94
September	97.8	109	65.0	54	81.4	83.9	49.5	.10	95
October	94.9	104	55.8	49	75.4	73.4	41.0	T.	98
November	76.1	91	47.7	38	61.9	61.9	37.5	T.	89
December	67.8	79	41.1	28	54.4	55.7	45.5	.12	78
Yearly	86.6	116	56.1	28	71.4	72.3	45.5	4.33	89.1

[a] Only the rainfall and mean monthly temperatures were taken at Chillicothe, the relative humidity and sunshine are as reported by the Weather Bureau Station at Abilene, Tex.; the other records are as reported at Quanah, Tex.

[b] Data from the Weather Bureau Station at Yuma, Ariz.

TABLE I.—*Weather conditions during 1915 and 1916*—Continued

PUYALLUP, WASH., 1915[a]

Month.	Temperature (° F.).						Mean relative humidity.	Rainfall	Percentage of actual to possible sunshine.
	Maximum.		Minimum.		Mean for month.	Normal for month.			
	Mean.	Absolute.	Mean.	Absolute.					
							Per cent.	*Inches.*	
January	44.7	55	33.2	18	37.5	38.1	85	5.87	21
February	49.8	59	37.0	25	42.6	40.4	84	3.41	19
March	57.9	74	41.6	29	48.7	44.2	76	2.34	41
April	60.9	74	43.7	31	51.4	48.9	71	3.93	53
May	63.6	79	47.3	36	55.0	54.4	71	3.42	37
June	68.3	88	50.9	40	59.4	59.4	72	.43	48
July	73.3	90	55.2	46	64.8	63.4	71	2.28	44
August	77.3	89	56.0	44	65.2	63.0	66	.00	58
September	67.0	76	50.8	35	57.6	57.6	74	1.11	45
October	60.5	68	46.4	33	52.4	50.6	79	3.43	27
November	48.0	55	38.1	26	42.2	44.1	86	9.46	10
December	47.2	60	36.7	20	40.6	40.3	82	9.26	20
Yearly	59.9	90	44.7	18	51.4	50.4	76.4	44.9	35.3

CHULA VISTA, CAL., 1916[b]

January	58.2	65	46.7	36	52.4	54.0	82	7.56	48
February	63.5	78	49.2	38	56.4	54.6	81	.66	66
March	65.8	81	52.5	43	59.2	56.2	78	.98	69
April	66.7	79	53.8	48	60.2	58.2	78	.01	76
May	65.8	72	55.9	50	60.8	60.8	75	.01	73
June	66.2	74	56.7	52	61.4	63.8	83	T.	62
July	69.1	74	60.8	58	65.0	66.9	84	.02	59
August	72.0	84	61.9	58	67.0	68.7	84	.01	71
September	69.1	80	59.6	56	64.4	66.9	87	.25	62
October	65.1	78	53.5	46	59.3	63.0	83	.87	69
November	65.0	74	48.0	39	56.5	59.0	74	.05	81
December	60.2	76	44.6	38	52.4	55.7	75	1.14	62
Yearly	65.4	84	53.6	36	59.6	60.7	80.3	11.56	66.5

[a] Data from the Weather Bureau Station at Tacoma, Wash.
[b] Data from the Weather Bureau Station at San Diego, Cal.

The most striking features brought out in Table I are the high percentage of sunshine and the consistently high temperatures, especially during the months of June, July, and August, at Bard, Cal., and in contrast with this the very low figures for these two climatic features at Puyallup, Wash. The mean maximum temperature for June, July, and August at Bard is considerably over 100° F. for each month. This, according to the figures obtained by Sachs for corn, would indicate an excess of heat units during a part of the day at least. At Puyallup, Wash., even the maximum temperatures do not reach the presumed optimum for sorghums, and the actual mean temperature for the growing season is about 30° below this optimum (Table II). This lack of heat units no doubt is largely responsible for the indifferent growth which the sorghums made at Puyallup.

In order that the main features of difference can be more easily comprehended, a summary of weather conditions for the growing season alone is brought together in Table II.

TABLE II.—*Synopsis of weather conditions during the growing seasons* [a] *at Chillicothe, Tex., Bard and Chula Vista, Cal., and Puyallup, Wash.*

Station and year.	Temperature (°F).						Average of mean relative humidity.	Total rainfall.	Percentage of actual to possible sunshine.
	Maximum.		Minimum.		Average of monthly means.	Average of monthly normals.			
	Average of means.	Absolute.	Average of means.	Absolute.					
							Per cent.	*Inches.*	
Chillicothe, Tex. (1915)	88.9	109	64.3	45	75.6	78.5	61.3	20.50	75.0
Bard, Cal. (1915)	97.9	112	65.3	39	81.8	82.9	40.1	[b] 1.54	93.4
Chula Vista, Cal. (1916)	68.1	72	56.8	39	62.4	64.9	82.4	[b] 1.20	68.4
Puyallup, Wash. (1915)	69.9	90	52.0	35	60.4	59.6	70.7	7.24	46.4

[a] At Chillicothe, Bard, and Puyallup the months of May, June, July, August, and September were considered as the growing season. At Chula Vista the data for July, August, September, October, and November were used to make up the averages in the table because the sorghums were planted there in the latter part of June.

[b] Irrigation water supplied to the crop as needed.

In 1915 the rainfall at Chillicothe during the growing season was much above and the temperatures somewhat below the normal. Conditions, except for a short period during July, were rather favorable for rapid growth.

The seasons at Bard do not vary much from year to year. The summer season is characterized by heat so extreme that it seems to have a retarding effect on the growth of the ordinary field crops. These high temperatures are accompanied by low atmospheric humidity. The rainfall is negligible, but an abundance of soil moisture is supplied through irrigation.

The seasons differ but little at Chula Vista from year to year, but 1916 happened to be about 2 degrees cooler than normal and this seemed detrimental to the sorghums.

The rainfall at Puyallup, which averaged about 1½ inches per month, seems rather inadequate, but in the four months preceding the period under consideration there were 15.55 inches of rainfall, which no doubt left the soil full of moisture. This fact, taken in consideration with the low temperatures and small amount of sunshine, makes it appear probable that there was no serious deficiency in soil moisture during the summer.

EXPERIMENTAL RESULTS

COMPARISON OF THE GROWTH OF SORGHUM VARIETIES UNDER DIFFERENT WEATHER CONDITIONS

A general comparison of the growth of Sumac, Red Amber, and Honey sorgos, Blackhull kafir, Dwarf milo, and feterita is given in Table III.

TABLE III.—*Comparison of the growth of sorghum varieties at Chillicothe, Tex., Bard and Chula Vista, Cal., and Puyallup, Wash.*

Variety and location.	Date planted.	Date emerged.	Date 90 per cent ripe.	Growing season.	Final height.	Diameter of stem.	Remarks.
				Days.	*In.*	*In.*	
Sumac sorgo:							
Chillicothe	May 21	May 25	Sept. 13	115	80	0.75	A normal vigorous growth.
Bard	Apr. 22	Apr. 27	Sept. 2	133	108	.75	Tall, vigorous growth.
Chula Vista	May 23	May 29	Oct. 31	161	68	.63	Fairly normal growth, not especially vigorous.
Puyallup	May 15	May 30	(a)	a 143	30	.....	October 5, growth low and spreading, leaves somewhat curled; not yet heading.
Red Amber sorgo:							
Chillicothe	May 21	May 25	Aug. 13	84	64	.63	Uniform growth.
Bard	Apr. 14	Apr. 20	Aug. 10	118	90	.69	Normal, vigorous growth.
Chula Vista	June 27	July 2	Oct. 31	126	90	.56	A typical, fine-stemmed growth.
Puyallup	May 15	May 30	.....	143	40	.....	A few heads in bloom on October 5. Growth somewhat spreading. Leaves curled and red on the edges.
Honey sorgo:							
Chillicothe	May 21	May 25	Oct. 6	138	96	1.00	Late, rank growth.
Bard	Apr. 14	Apr. 20	Aug. 31	139	99	.75	A good, vigorous growth, some tendency to lodge.
Chula Vista	June 28	July 3	Nov. 12	137	92	.75	Medium height. growth normal.
Puyallup	May 15	June 1	.....	143	11	.....	A low, spreading inferior growth. No heads.
Blackhull kafir:							
Chillicothe	May 21	May 25	Sept. 10	112	62	1.00	Normal, vigorous growth.
Bard	Apr. 16	Apr. 21	Sept. 2	139	72	.81	Good, vigorous growth.
Chula Vista	June 28	July 3	Dec. 5	160	74	.94	Uniform growth and well-filled seed heads.
Puyallup	May 15	May 29	.....	143	33	.....	October 5, growth quite upright, but with no heads.
Dwarf milo:							
Chillicothe	May 21	May 25	Aug. 30	101	50	.75	Uniform, vigorous growth.
Bard	Apr. 22	Apr. 27	Aug. 30	130	58	.56	Typical milo of poor forage value, but good grain.
Chula Vista	June 29	July 4	Nov. 5	129	50	.50	Typical milo, but with rather numerous tillers.
Puyallup	May 15	May 29	.....	143	27	.....	October 5, beginning to head, but very undersized.
Feterita:							
Chillicothe	May 21	May 25	Aug. 13	84	58	.63	Uniform, vigorous growth.
Bard	Apr. 20	Apr. 25	Aug. 12	114	65	.50	Stems too slender for typical milo.
Chula Vista	June 29	July 4	Nov. 10	134	66	.69	Fair growth, but did not seem at home under these conditions.
Puyallup	May 15	May 29	.....	143	18	.....	October 5, growth spreading, leaves curled and red on edges, no heads.
Average:							
Chillicothe	.....	.....	.....	106	68	.79	
Bard	.....	.....	.....	129	82	.68	
Chula Vista	.....	.....	.....	141	73	.68	
Puyallup	.....	.....	.....	.....	27	.....	

[a] None of the varieties matured at Puyallup, so that the growing season for each variety represents only the period from the date of planting to October 5.

All of the varieties under discussion are thoroughly at home under the climatic conditions at Chillicothe. A long experience in growing sorghum at this point indicates that Chillicothe's climate is well suited to the requirements of the crop and the results there may be taken as a criterion of normality of growth (Pl. 11, 12). The data in the table show an average growing season for the varieties under consideration of 106 days at Chillicothe, as compared with 129 days at Bard, and 141 days at Chula Vista. We find, however, in Table V that a later date of plant-

ing at Bard, will shorten the growing season 21 days, thus making the normal growing season at Bard 108 days, which is about the same as at Chillicothe. This is assumed as the normal growing season for Bard, since the growth of sorghums planted at this date is more nearly normal than when planted earlier.

The longer growing season at Bard and Chula Vista seem to have resulted in an increase in height most noticeable at Bard, where the plants averaged 14 inches taller than at Chillicothe. Again, however, we find by consulting Table V that where the sorghums were planted later and the growing season was shortened the height was reduced an average of 8 inches. This would make the average height for Bard 74 inches, only 6 inches taller than at Chillicothe. At Chula Vista the average height was 73 inches, which is but little over normal. The average height of 27 inches at Puyallup emphasizes the very inferior growth which the sorghums made under the conditions existing there.

In diameter of stem the climatic effect is the reverse of that where height is concerned. The stems were larger and stronger at Chillicothe. than at either Bard or Chula Vista. If we take into consideration the effect of the time of planting at Bard, however, the difference in diameter at Chillicothe and Bard is very slight, only 0.02 inch.

On the whole Table V shows that a normal growth may be expected at both Chillicothe and Bard if the time of planting at the latter point is properly regulated. At Chula Vista the rather low temperatures lengthened the growing season very markedly and caused the plants to have somewhat slender stems. At Puyallup, where low day temperatures were combined with a very decided deficiency in the sunshine and with cool nights the sorghums did not behave normally, and all failed to mature.

At Puyallup measurements were taken every two weeks on the growth made by the sorghum varieties, and these measurements emphasized the backwardness of the growth. For Sumac sorgo the growth was for the first month, 4 inches; second month, 3 inches; third month, 10 inches; and fourth month, 13 inches. Blackhull kafir grew in the first month only 4 inches: second month, 4 inches; third month, 12 inches; and fourth month, 12 inches. Dwarf milo, which was the only variety that headed, made a growth of 5 inches the first month; 2 inches the second month; 12 inches the third month; and 9 inches the fourth month. This low rate of growth indicates a temperature too low to stimulate properly the growing functions of sorghums, or else the deficiency of sunshine had a very decided retarding effect on their growth. The percentage of possible sunshine was only 46 at Puyallup, while it was 68 at Chula Vista.

The total degrees of positive temperature[1] received by the sorghums at Puyallup was 1,615° F., at Chula Vista, 1,895°, at Bard, 4,236°, and at Chillicothe 3,028°. The difference between the total seasonal heat units at Chula Vista and Puyallup is rather small, and it is difficult to believe that this difference in heat units is alone responsible for the failure of the sorghums to reach the heading stage at Puyallup, while they matured perfectly at Chula Vista.

Evidently there was an excess of heat at Bard above what the plants could utilize, since they ripened at Chula Vista with 2,341° F. and at Chillicothe with 1,208° less positive heat units than were available at Bard during the period covered by their growth. The old theory of botanists that a given total of heat units will produce the same phase of vegetation regardless of latitude, longitude, or local climatic conditions is thus disproved, but the results do substantiate Linsser's law of growth as described by Abbe (*1, p. 214*).

This law can be stated as follows: In two different localities the sums of positive daily temperatures for the same phase of vegetation is proportional to the annual sum total of all positive temperatures for the respective localities—that is, the heat required in any locality to produce a given phase of development in vegetation bears a constant ratio to the total positive heat units available in that place. This ratio has been styled the "physiological constant." If 50° F. is considered as the minimum temperature for growth in the sorghums, the yearly total of positive heat units at Chillicothe in 1915 was 5,618° F.; at Bard, 1915, 7,989°; and at Chula Vista, 1916, 3,600°. The positive heat units required to bring the sorghums to maturity at Chillicothe were 3,028° F., at Bard, 4,236°, and at Chula Vista 1,895°. It appears, therefore, that the physiological constant of sorghum for the period from planting to maturity is about 0.53. The conformance of the sorghums in these three cases to Linsser's law is rather remarkable, the exact ratio in each case being as follows:

Chillicothe	3,028:5,618, or 0.539
Bard	4,236:7,989, or .530
Chula Vista	1,895:3,600, or .526

Although Linsser's law seems to furnish a rule for the behavior of sorghums in respect to temperature, it does not take into account the effect of sunlight and other factors, which are also important.

The data for a more exact comparison of the growth attained by certain varieties of sorghum at Bard and Chula Vista have been assem-

[1] These totals were calculated according to the second method of Angot described by Abbe (*1, p. 79*), except that 50° F. was taken as the zero point of growth instead of 43° F. Sachs (*2*) determined the minimum temperature for corn as 49.1° F., and as sorghum is notably more sensitive to low temperatures than corn, 50° F. was chosen arbitrarily as the minimum temperature for growth, and only those temperatures above 50° F. were used in compiling the totals. For Chula Vista, Bard, and Chillicothe the totals are based on the average season required by the sorghums for maturity. At Puyallup it includes the period from the date when the sorghums were planted until the first killing frost in the fall.

TABLE IV.—*Comparison of the growth of sorghums at Bard and at Chula Vista, Cal.*

Variety.	Serial No.	Date planted.		Growing season.			Stems.						Leaves.									Panicle.					
							Height.			Diameter.			Number.			Length.			Width.			Length.			Diameter.		
		Chula Vista.	Bard.	Chula Vista.	Bard.	Excess, Chula Vista.	Chula Vista.	Bard.	Excess, Bard.	Chula Vista.	Bard.	Excess, Bard.	Chula Vista.	Bard.	Excess, Bard.	Chula Vista.	Bard.	Excess, Bard.	Chula Vista.	Bard.	Excess, Bard.	Chula Vista.	Bard.	Excess, Bard.	Chula Vista.	Bard.	Excess, Bard.
		1916.	1916.	*Days.*	*Days.*	*Days.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*				*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*	*In.*
White African sorgo	1546	June 27	June 14	136	120	16	111	112	1	0.75	1.19	0.44	12	17	5	31	25	−6	3.25	3.5	0.25	7	7.5	0.5	2.75	5	2.25
Blackhull kafir	17569	June 28	...do....	160	120	40	74	68	−6	.94	.94	.00	13	16	3	31	25	−6	4.25	4	−.25	9.5	10.0	.5	3.25	2.75	−.5
Dwarf hegari	34911	May 24	...do....	121	121	0	42	60	18	.38	.88	.5	7	19	12	20	34	14	2	3.5	1.5	6.5	7.5	1.0	1.75	3.25	1.5
Brown kaoliang	38199	June 30	June 15	135	108	27	72	78	6	.5	.5	.00	10	10	0	22	26	4	2.75	2.25	−.5	7	7.5	.5	2.5	3.5	1
Average						20.75			4.75			.24			5			1.5			.25			.63			1.06
			1915.																								
Red Amber sorgo	17548	June 27	Apr. 14	126	118	8	90	90	0	.56	.69	.13	8	10	2	22	30	8	2.5	3.25	.75	12	12	.00	9.5	3.5	−6
Sumac sorgo	17554	May 23	...do....	161	164	−3	64	108	44	.75	.94	.19	12	18	6	29	33	4	3.25	3	−.25	6.5	6.5	.00	2.25	2	−.25
Dwarf milo	18684	June 29	Apr. 19	132	106	26	40	59	19	.5	.56	.06	6	8	2	23	26	3	2.25	2.75	.5	7	6.5	−.5	3.5	2.25	−1.25
Planter sorgo	18930	June 28	Apr. 14	156	123	33	88	92	4	.63	.75	.12	11	12	1	30	32	2	3.5	3.5	.00	8.25	9	.75	3.25	4.5	1.25
Feterita	19517	June 29	Apr. 20	136	118	18	70	70	0	.69	.56	−.13	9	9	0	27	24	−3	3.25	2.75	−.5	6.5	8	1.5	3.25	4	.75
Red kafir	19742	May 23	Apr. 16	160	182	−22	74	78	4	.63	.94	.31	10	17	7	27	32	5	3.25	3	−.25	12	12	.00	2.25	2.5	.25
Collier sorgo	19770	...do....	Apr. 20	168	176	−8	95	126	31	.75	1.06	.31	11	17	6	32	37	5	3.5	3	−.5	12.5	9.5	−3	5.5	.....	.....
Florida sorgo	20813	June 28	Apr. 14	167	154	13	100	112	12	.81	1.06	.25	14	16	2	27	34	7	3	3.25	.25	7.5	10	2.5	2.75	2.25	−.5
Freed sorgo	29160	...do....	...do....	133	117	16	73	66	−7	.38	.38	.00	6	7	1	25	24	−1	3	2.5	−.5	12	11	−1	7.5	7	−.5
Orange sorgo	32889	May 23	May 17	161	142	19	76	96	20	.69	1	.31	12	16	4	34	36	2	3.75	3.5	−.25	10.75	10	−.75	3.5	2.5	−1
Gooseneck sorgo	32893	June 28	Apr. 15	137	153	−16	88	88	0	.63	.63	.00	10	12	2	26	27	1	3.25	3.25	.00	7	10	3	3	4.5	1.5
Standard milo	35048	June 29	May 17	139	108	31	63	80	17	.5	.56	.06	7	7	0	30	25	−5	3.25	2.25	−1	7	7	.00	3.5	2.5	−1
Average						9.58			12			.13			2.75			2.33			−.15			.21			−.61

bled in Table IV. The comparison of weather conditions at these two points, both of which are in southern California, is available in Tables I and II.

It will be noted in Table IV that most of the data from Bard are for the year 1915, while those for Chula Vista are for 1916. The weather conditions at Chula Vista and Bard are so uniform from year to year that the comparison has practically the same value as if all the plantings had been made in 1915 at both places. The greatest factor of uncertainty in the tabulated data is the effect of the date of planting. At Bard in 1915 most of the numbers were planted about the middle of April, while in 1916 the plantings were not made until the middle of June. The effect which this early planting had on the sorghums is brought out more fully in Table V. It can be said here, however, that the later planted sorghums were more nearly normal, especially in the seed parts.

Table IV gives a comparison not only of the growing season and height, but also of the number, length, and width of the leaves, and the length and diameter of the panicles. For the varieties which were planted at both places in June the magnitude of all characters was greater at Bard than at Chula Vista and the growing season was shorter. This difference of 21 days in the growing season is perhaps a fair measure of the effect of the low temperatures and reduced actinic value of the sun's rays at Chula Vista. High fogs obscure the sun quite often for hours in the morning and evening, and the temperature is seldom above 70° F. for any considerable part of the day. At Bard, on the other hand, desert conditions prevail, the sky is usually cloudless and the sunlight is intense, while the temperatures reach a maximum of over 100° F. day after day. Considering only the three varieties White African sorgo, Blackhull kafir, and Brown kaoliang which were planted at Bard on June 14, 1916, and at Chula Vista only two weeks later, the sum total of the positive temperatures received from planting date to maturity was at Bard 4,301° F. and at Chula Vista 1,923° F. It is evident, therefore, that although the growing season averaged 28 days shorter at Bard there was available for the plants' use a much larger number of heat units than at Chula Vista. These conditions resulted in taller, slightly larger stems, and a greater number of leaves which were both longer and wider, except in the early planted sorghums at Bard which had slightly narrower, though longer leaves than at Chula Vista. The panicle was larger at Bard, especially in the sorghums which were planted at both places in June.

The most remarkable variation which took place, however, is in the number of leaves, a character that has heretofore been considered relatively stable. All the plantings were made with seed from bagged heads, so the variation can not be attributed to cross-pollination. Dwarf hegari, which exhibits an extreme variation of 12 leaves, is notoriously

variable, but Blackhull kafir 17569, Sumac sorgo 17554, and most of the other varieties listed are standard strains which are known to be stable. Blackhull kafir showed a difference of 3 in the number of leaves under the differing conditions, and Sumac sorgo had 6 more leaves at Bard than at Chula Vista. Evidently unfavorable conditions of growth cause a reduction in the number of leaves and this character is, therefore, of uncertain value as a basis of classification or botanical description.

Table IV is even more interesting for the study of individual varieties than it is in a study of the average variation. Thus, Dwarf hegari, Dwarf and Standard milos, and Sumac, Collier, Florida, and Orange sorgos show a striking difference in height under different weather conditions, while the other varieties vary much less in height, but fully as much or more than above-named sorts in the size of the leaf and panicle.

EFFECT OF THE DATE OF PLANTING ON THE GROWTH OF SORGHUMS

The effect of different dates of planting on the growth of sorghum plants is set forth in Table V. Other things being equal, this effect apparently is correlated with the presence of high temperatures at different periods of the life cycle. In 1915, when the plantings were made in April, the early stages of growth took place in a period of moderate temperatures, and the flowering and fruiting functions were carried on in a period of very high temperatures. In 1916 the plantings were made about the middle of June. Growth began during a period of high temperatures and maturity took place when conditions were more moderate.

A study of Table I in connection with Table V shows the relation that the period of high summer temperatures bears to the growing periods of each planting. The conditions in 1916 were practically the same as in 1915. A comparison of the mean temperatures with the normals shows how uniform the seasons are at Bard.

An examination of Table V shows that deferring the planting date 55.5 days shortened the growing season for the 12 varieties under test an average of 21 days. In spite of the shorter growing season in 1916 the total of positive heat units available during the period of growth in the two years was practically the same, 4,628° F. in 1915 and 4,318° in 1916. The early date of planting and longer growing season produced taller plants and longer leaves, but in all other characters the excess in magnitude was with the later planting. On the whole, the plants from the later planting were more nearly normal in their growth and produced a better seed crop. It would appear, therefore, that more favorable conditions of growth are obtained if the date of planting is regulated so that the early stages of the plant's development coincide with a period of high temperatures and the later stages, when the plant is nearing maturity, come when moderate temperatures prevail.

TABLE V.—*Comparison of the growth of sorghums when planted on different dates at Bard, Cal.*

Variety.	Serial No.	Date planted.			Growing season.			Stems.						Leaves.									Panicle.					
								Height.			Diameter.			Number.			Length.			Width.			Length.			Diameter.		
		1915	1916	Difference in dates.	1915	1916	Excess, 1915.	1915	1916	Excess, 1915.	1915	1916	Excess, 1916.	1915	1916	Excess, 1916.	1915	1916	Excess, 1915.	1915	1916	Excess, 1916.	1915	1916	Excess, 1916.	1915	1916	Excess, 1916.
				Days.	*Days*	*Days*	*Days*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*				*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*	*Ins.*
White African sorgo	1546	May 15	June 14	30	143	120	23	114	112	2	0.75	1.19	0.44	16	17	1	30	25	5	3	3.5	0.5	6.5	7.5	1.00	2.75	5	2.25
Shallu	2650	Apr. 22	June 15	54	142	119	23	110	102	8	.75	.75	.00	14	15	1	36	34	2	3.25	3.5	.25	12.0	12.0	0	7	7	.00
Blackhull kafir	17569	Apr. 16	June 14	59	139	120	19	72	68	4	.81	.94	.13	16	16	0	34	25	9	3.75	4	.25	10	10	0	2.5	2.75	.25
Red kafir	19751	Apr. 16	June 14	59	136	118	18	108	95	13	.75	.88	.13	16	13	— 3	36	29	7	4	3.5	— .5	9	9.75	.75	1.75	2	.25
Red kafir	19756	Apr. 16	June 14	59	139	114	25	108	95	13	.88	1.31	.43	16	15	— 1	34	28	6	3.5	4	.5	11	11	0	2.5	3.75	1.25
White kafir	19764	Apr. 16	June 14	59	145	119	26	122	107	15	.75	.88	.13	18	17	— 1	33	26	7	3.5	3.88	.38	10	9	—1	3.5	4.75	1.25
White kafir	19765	Apr. 16	June 14	59	149	120	29	98	74	24	.81	1	.19	17	14	— 3	35	27	8	3.5	4	.5	12	12	0	2.25	5	2.75
Pink kafir	19906	Apr. 16	June 14	59	143	120	23	94	66	28	.88	.88	.00	18	15	— 3	35	28	7	3.5	3.88	.38	11.5	12	.5	1.5	2.25	.75
Blackhull kafir	22653	Apr. 16	June 14	59	167	119	48	78	67	11	1	.88	— .12	19	15	— 4	33	29	4	4	3.75	— .25	11.5	12.5	1	2.75	3	.25
Red milo	24963	Apr. 19	June 14	56	113	111	2	70	80	—10	.56	.69	.13	9	13	4	27	25	2	3	3.25	.25	7.5	7	— .5	2.5	3.75	1.25
Dwarf hegari	34911	Apr. 17	June 14	58	117	121	— 4	53	60	— 7	.56	.88	.32	8	19	11	25	34	—9	2.5	3.5	1	8.5	7.5	—1	2	3.25	1.25
Brown kaoliang	38199	Apr. 21	June 15	55	127	108	19	74	78	— 4	.69	.5	— .19	12	10	— 2	24	26	—2	3	2.25	— .75	7	7.5	.5	3.5	3.5	0
Average				55.5			21			8.08			.13			0			3.83			.13			.10			.96

SUMMARY

(1) Sorghum is semitropical in its adaptations and does not thrive in regions of low temperatures.

(2) Sunshine is probably an important factor of growth; witness the difference of growth at Chula Vista, Cal., and Puyallup, Wash., where the mean temperatures and the total positive heat units available are but little different.

(3) The "physiological constant" for the ripening phase of sorghums according to Linsser's law of growth is about 0.53.

(4) Extremely high temperatures during the period of flowering and fruiting result in a decreased yield of seed.

(5) The date of planting should be so arranged that germination and early growth of the plants will take place during the period of high temperatures and the flowering and fruiting when more moderate temperatures prevail.

(6) Adverse weather conditions affect such supposedly stable characters as the number of leaves per plant, as well as the volume of growth.

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PLATE 11

A typical plant of Blackhull kafir grown at Chillicothe, Tex.

PLATE 12

A typical plant of Dwarf milo grown at Chillicothe, Tex.

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OVERWINTERING OF THE HOUSE FLY

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INTRODUCTION

Experiments and observations bearing on the problem of the method of the overwintering of the house fly (*Musca domestica* Linnaeus) were begun by the Bureau of Entomology during the fall of 1914 at the Arlington Experimental Farm of the Bureau of Plant Industry. The following spring the work was transferred to the experiment station of the Bureau of Animal Industry at Bethesda, Md., and was continued there during the two seasons 1915–17.

OVERWINTERING OF ADULT FLIES UNDER EXPERIMENTAL CONDITIONS

UNDER OUTDOOR CONDITIONS

A large number of experiments were conducted during the winter of 1914–15. Reared flies were put in screen-wire cages, which measured 10 by 12 by 22 inches. In the bottom of each was a drawer in which food and water were supplied. The food materials usually consisted of sliced banana and fresh horse manure. The cages were kept on shelves of a screened insectary where they were subject to outdoor temperature and humidity, but were protected from rain and to some extent from direct sunlight. The banana and manure were renewed at frequent intervals so that a fresh food supply was always available. The most significant results of these experiments are summarized in Table I.

[1] The writer wishes to express his appreciation of the kindness of Mr. E. C. Butterfield, superintendent of the Arlington Experimental Farm near Rosslyn, Va., and Dr. E. C. Schroeder, superintendent of the experiment station of the Bureau of Animal Industry at Bethesda Md., for the facilities afforded during the course of the work. The writer's thanks are also due to Dr. C. H. T. Townsend, of the Bureau of Entomology, for determining many specimens of Muscidae, and to Mr. Frederick Knab, of the Bureau of Entomology, for the determination of many nonmuscids.

TABLE I.—*Longevity of house flies during winter in cages under outdoor conditions*

Experiment No.	Date of emergence.	Last flies died.	Maximum longevity.	Temperature. Min.	Temperature. Max.	Remarks.
	1914.	1914.	*Days.*	°*F.*	°*F.*	
M-1.....	Oct. 18	Nov. 27	40	18	79	Minimum temperature of 18° F. occurred November 24.
M-2.....	Nov. 12	Dec. 14	32	18	67	
M-3.....	Nov. 15-17	Dec. 15	28-30	10	67	Minimum of 10° F. occurred on December 15, 1914, a. m. All appeared dead but were kept in insectary till Feb. 12, 1915, then taken to greenhouse, but none recovered after several days in a warm room.
M-7.....	Nov. 27	Dec. 15	18	10	64	Minimum temperature of 10° F. occurred December 15, 1914, a.m. No recovery after several hours in warm greenhouse.
M-8.....	Nov. 27	Dec. 15	18	10	64	
3-A.....	Nov. 6	Dec. 4	28	20	73	
4-A2.....	Nov. 3	Dec. 4	32	18	79	
5-A1.....	Nov. 2	Nov. 21	19	20	79	
5-A2.....	Nov. 12	Nov. 30	18	20	67	
5-A3.....	Nov. 2	Dec. 1	29	18	79	
5-A4.....	Nov. 7	Dec. 4	27	18	73	
11-1.....	Nov. 11	Dec. 14	32	18	64	
	1915.	1915.				
M-20.....	Mar. 2	Mar. 13	11	23	53	Reared in greenhouse and given time for one feeding before being taken outdoors. Continued cold prevented any feeding after removal to insectary.

In these and other experiments it was found impossible to keep house flies alive during the winter in cages under outdoor conditions in spite of the presence of food and shelter afforded by the mass of food material and the corners of the wooden framework of the cages. It was found that the first really cold night of the winter (December 15), when the temperature fell as low as 10° F., proved fatal. No flies revived after exposure to this temperature even when kept several hours or even days in a warm greenhouse. On the other hand, after exposure to temperatures of 22° or 25°, a large percentage often revived. Continued exposure to low temperatures which interfere with normal activities, especially feeding, will eventually prove fatal, as was the case in experiment M-20, Table I. Since at this latitude temperatures often fall below 10° during the winter, the foregoing experiments point to the conclusion that house flies in the adult state can not pass through the winter when exposed to outdoor conditions.

IN PROTECTED OR SLIGHTLY HEATED LOCATIONS

An attempt was made to test experimentally the prevailing idea that house flies can pass the winter in cracks and crevices in protected places of houses and stables. A portion of one of the barns of the Arlington farm was selected. The barn is a two-story structure, the first floor of which has stalls for 25 horses, a carriage room, and harness room. On the second floor are storage spaces for hay and grain, and at one end over the carriage room is the farm office. Steam pipes conducting heat to this office pass under the concrete floor of the carriage room and up inside the wall of the harness room. The carriage room has large doors on the south side and three windows on the north. A certain amount of heat is given off from the steam pipes in the harness room and through the floor so that the temperature is modified and does not fluctuate as much or as rapidly as does the outdoor temperature. A shelf was constructed in the carriage room about 5 feet from the floor and some distance away from both the steam pipes and the windows. Fly cages were kept on this shelf, and a thermograph was installed.

Of several experiments conducted here the following two gave the best results:

EXPERIMENT No. 13.—Sixty-five flies (45 males and 22 females), which had been reared in the greenhouse, emerged between December 26 and December 28, 1914. They were transferred to wire cages and supplied with banana and a mixture of bran and fresh horse manure well moistened. This material was renewed from time to time as needed. The cage was put on the shelf in the stable on December 29 and was examined every other day after this date. The flies died, a few at a time, up to March 9, 1915, when the last ones (9 males and 8 females) were found dead. In this experiment, then, 17 flies had been kept alive for a period of from 70 to 72 days. The maximum temperature during this period was 62° and the minimum was 29°. The mean temperature for the entire period was 43.8°.

EXPERIMENT No. 16.—From rearing experiments in the greenhouse 46 flies emerged between January 28 and February 3, 1915. These were transferred to a cage with the same kind of food as in Experiment No. 13, and placed on the shelf in the stable on February 3. The last fly of this lot, a male, was found dead on March 9, 1915, giving a maximum longevity of from 41 to 47 days. They were subjected to the same temperature conditions as obtained in experiment 13.

The following season, experiments of a similar kind were conducted in the attic of the apicultural laboratory of the Bureau of Entomology at Drummond, Maryland. This attic has one large south room which is plastered and heated. The heat was turned off and the door communicating with the unplastered part was left open. Cages were kept on a table in this room and the flies were supplied with banana and a sponge soaked in a sugar solution. Maximum and minimum thermometers were used in recording temperatures.

The best results were obtained in experiments 60 and 61 following:

EXPERIMENT No. 60.—House-fly puparia were collected on November 11, 1915. Twenty flies, emerged between November 13 and 16, were transferred to a cage and

placed on the table in the attic. Fresh food was supplied as needed, and a daily watch was kept. The last flies of this lot died on January 4, 1916, thus living from 49 to 52 days.

EXPERIMENT No. 61.—A similar experiment was conducted with flies which had emerged on November 17 to 19, 1915. The last flies died on January 10, 1916. This gives a longevity of from 52 to 54 days. The maximum temperature during this period was 76°, the minimum 35°, and the mean, 56.9°.

These experiments with caged flies in protected or slightly heated locations gave longevity records of 41, 47, 49, 52, 54, and 70 days. Dove (5) [1] records an experiment at Dallas, Texas, in which a fly was kept alive for 91 days in a large cage kept in an unoccupied room. In the latitude of Washington it would be necessary for flies to live at least from the first week of December (the time of the last emergence in the fall) until early in April (when temperatures first are high enough to induce oviposition) and be in condition to oviposit at the end of that time.

IN HEATED BUILDINGS

Overwintering experiments were also carried out in one of the warmest rooms of the greenhouses at the Arlington Experimental Farm. The room used was one in which tomatoes were grown, with temperatures ranging as a rule from a minimum of 55° or 60° at night to about 80° during the day. The humidity also was usually high. A few lots of flies reared in experiments during the fall of 1914 were transferred to this greenhouse at times during October and November. These flies were kept in cages already described and supplied with banana and fresh horse manure or a mixture of bran and horse manure. Eggs were readily obtained except when the fungous disease *Empusa muscae* killed off the adults too rapidly, and from the original lots of flies several generations were reared during the course of the winter. Table II gives the principal results of the experiments bearing upon the question of how long the flies will live in heated rooms during the winter.

In these experiments, where caged house flies were kept in a warm humid atmosphere during the winter, longevity records of from 9 to 40 days were obtained. It will be noted from Table II that the short records are all due to the attacks of *Empusa muscae*. There is, however, no evidence that adult flies, even if they escape the attacks of the fungus, can survive the winter in heated buildings. They were kept alive much longer in places such as attics and stables, which were only slightly heated.

[1] Reference is made by number to "Literature cited" pp. 168–169.

TABLE II.—*Longevity of house flies during the winter, in a heated room in a greenhouse*

Experiment No.	Flies emerged.	Last fly died.	Maximum longevity.	Remarks.
	1914.	1914.	*Days.*	
M-4.....	Nov. 18	Nov. 29	11	Fungus appeared on sixth day after emergence, and killed all flies.
M-5.....	Nov. 19	Nov. 30	11	Two dead on third day. Fungus appeared first on eighth day after emergence, and killed all remaining flies.
M-6....	Nov. 21	Dec. 4	13	Fungus first appeared on sixth day after emergence.
M-8....	Nov. 28	Dec. 12	14	Fungus appeared first on sixth day after emergence, and was cause of all deaths.
M-11...	Dec. 2	Dec. 15	13	Fungus appeared on eighth day and caused all subsequent deaths.
		1915.		
M-14...	Dec. 30	Feb. 8	40	No deaths from fungus, although the cage had previously held diseased flies and had not been disinfected.
	1915.			
M-17...	Feb. 7	Mar. 1	22	No fungus noted.
M-18...	Feb. 26	Mar. 20	20	Do.
M-21...	Mar. 3	Mar. 31	28	Do.
5-A5...	Nov. 7	Nov. 23	16	Fungus first appeared on tenth day, and was cause of all deaths.
5-A6...	Dec. 4	Dec. 18	14	Fungus appeared on the sixth day and was cause of all deaths.
5-B1...	Dec. 9	Dec. 28	19	Fungus appeared on the fourteenth day.
5-B2...	Dec. 13	Dec. 31	18	Fungus appeared on the tenth day.
5-B3...	Dec. 16	Dec. 31	15	No fungus noted.
11-A1..	Nov. 25	Dec. 4	9	Severe fungus attack, beginning on sixth day after emergence, was cause of all deaths.

Incidentally the experience with *Empusa muscae* in these experiments brought out the following points: (1) All the flies which emerged before December 30, with one exception (see lot No. 5-B3, Table II) were attacked by the fungus. All the lots of flies which emerged after December 30 gave no evidence of fungus attacks, although kept under the same conditions in cages which, earlier in the year, had held diseased flies, and which subsequently had not been disinfected. (2) The shortest time between the emergence of the flies and the first death from *Empusa muscae* was 6 days, but all were not affected at the same time; in some cases the period being extended to 19 days. It was found possible to obtain eggs from nearly every lot of flies before the fungus finally destroyed them. Even if it were possible to propagate *Empusa muscae* artificially and to disseminate the spores where they would be taken up by flies, it is doubtful whether it would be entirely effective, since, as the records show, eggs were often obtained before all the flies had been killed. (3) Experiments with the house fly do not support Dove's theory, based on experiments with Lucilia, that the fungus develops principally in sexually mature and in fertilized flies, which do not oviposit on account of low temperatures or in the absence of media for deposition.

OVERWINTERING OF ADULT FLIES UNDER NATURAL CONDITIONS

Frequent observations were made during the winter on the occurrence and behavior of house flies found living under natural conditions. For convenience in comparison with the experimental results these notes are summarized under the same headings.

UNDER OUTDOOR CONDITIONS

In the fall of 1914 observations were begun early in November. Adult house flies were found outdoors during warm days up to December 9. On December 1 several house flies were collected at a compost heap on the Arlington farm, and again on December 3. On this same date several recently emerged flies were taken near a heap of pig manure at the Bethesda farm. On December 9 many recently emerged flies were found on a heap of horse manure at College Park, Md., but after this date none were found outdoors until April, 1915, when the following notes were made:

April 7. No *Musca domestica* taken. Collected *Pollenia rudis* Fabricius, *Phorbia cinerella* Fallén, Sepsidae, and Borboridae.

April 8. Swept from horse dropping in road *Lucilia sericata* Meigen, *Orthellia cornicina* Fabricius, *Myospila meditabunda* Fabricius, *Phorbia cinerella* Fallén, *Copromyza equina* Fallén, and an undetermined species of Scatophaga.

April 10. Air temperature, 80° F., at 2 p. m. Search was made at pigpens, stables, manure heaps, etc. No *Musca domestica* found. *Muscina stabulans* Fallén was collected.

April 16. No *Musca domestica* found. Collected *Pollenia rudis*, *Phormia regina* Meigen, *Lucilia sericata*, and others.

April 25. A few house flies were taken by Max Kisliuk near stable at College Park, Md.

April 30. One female house fly taken near stable at the Arlington farm.

During the winter of 1915–16 flies were found outdoors as late as December 4. On that date one house fly was seen on wall on the south side of one of the animal houses of the Bethesda Experiment Station. No house flies were again found outside until the following April. Several flytraps were set out at various places on the Bethesda station, some on March 30 and others April 6, and were kept in operation throughout the season. Many species were caught, including *Pollenia rudis*, *Phormia regina*, *Muscina stabulans* Fallén, and *M. assimilis* Fallén, from the beginning of the exposures, but *Musca domestica* put in its first appearance on April 20, 1916.

The records for the winter of 1916–17 are similar. House flies were collected as late as December 12, 1916, after which none were again taken until the following spring. A few house flies put in an appearance at a very early date this year. Two females and one male were taken in a flytrap on March 26, 1917. A few scattered specimens were taken at various times during April. The unusually early appearance of house flies outdoors at this date was doubtless due to the fact that breeding

had continued and flies were present all winter in the animal house (see pp. 156–157) and that they escaped from the house on mild days during March and early April.

It will be noted that these observations on wild flies under outdoor conditions agree very closely with the experimental results with caged flies. Uncaged flies disappeared at about the same time that the caged flies were killed by the cold in early December.

UNHEATED BUT PROTECTED SITUATIONS

A very favorable unheated but protected location was found in one of the stables of the Arlington farm. This stable was at the west end of a long building and was protected on the north and east by a stone wall and high ground. The entrance was at the west and the ceiling was low and tightly boarded. Flies were collected at frequent intervals as late as January 19, 1915. Many of these were found with the ptilinum still exserted, showing that they were freshly emerged. Puparia were found in an accumulation of material under one of the feed troughs, and from them flies were emerging. All flies completely disappeared, however, after January 19 and none were again taken here until April 30, 1915.

At Bethesda, Md., no house flies were taken in unheated stables from December 7, 1915, till April 27, 1916. During the winter of 1914–15 collections were made in the attic of a dwelling house on the Arlington farm, and during the next two winters a careful watch was kept in the attics of one or two dwelling houses and of the apicultural laboratory at Drummond, Md. No house flies were found in such locations, although *Pollenia rudis* was present throughout the entire winter each season.

The experimental results showed that attics and slightly heated stables and similar places offer the most favorable temperature conditions conducive to longevity. Yet observations indicate that house flies never select such places for spending the winter. The records given by Copeman and Austen (*4*) of flies sent in to them from various places in England include no specimens of house flies taken in dormant or inactive condition in attics or other unheated places. It is shown that they "were all taken in an active condition" and from heated locations, such as dining rooms, kitchens, or bake shops.

IN HEATED BUILDINGS

Copeman and Austen (*4*) pointed out that—

> under the exceptionally favorable conditions afforded by confectioners' shops and restaurants, house flies of both sexes may survive in some numbers, at any rate until February.

But there is—

> nothing in the shape of proof that female house flies found alive at the end of the winter actually survive until oviposition takes place. * * * The ultimate fate of these insects is however at present unknown.

By restricting observations to certain selected places and by continuing them throughout the entire season the writer has been able to collect data which, it is believed, offer an explanation of the presence of flies during the winter months and of their ultimate fate. The following paragraphs bear on this point.

Some observations were made at a boarding house near Washington during the winter of 1915–16. There seemed to be complete indifference on the part of the occupants to the presence of flies, and the writer, in order to make some observations on these flies under natural conditions, was careful not to arouse any enthusiasm for fly killing. House flies were present in kitchen and dining room, and occasionally in bedrooms, throughout the fall and early winter. Their numbers gradually decreased as winter set in, but a few stragglers were noted and watched well into January. The last of them (two males) were noted in bedrooms on January 31. None were again seen in this house until the following May, some time after they had first appeared outdoors.

At the Bethesda station a small building is used daily for baking a coarse bread from meat scraps and corn meal to be fed to dogs. Temperatures were always high during the day, but the fires were allowed to go out during the night, and at such times the temperature often fell below freezing. House flies were present in large numbers during the fall. *Empusa muscae* carried off large numbers of them, but, in spite of this, some persisted during December and well on into January. There was a gradual and steady decrease and the last ones were observed on January 27, after which none were again seen here until the following May.

In both the above cases the flies had free access to food, but there was hardly any opportunity for oviposition. Their behavior was quite normal—that is, when the buildings were heated, they were active, invading everything with their usual annoying persistence, and when the temperatures were low they could be found at rest on the ceiling or walls, especially in the corners.

During the winter of 1916–17 special attention was paid to the conditions existing in the animal house at Bethesda, where large numbers of rabbits and guinea pigs are kept. The building used for this purpose is a long two-story structure, the walls of the first story being of masonry and the second story of frame construction. The floor of the first story is below ground level, and on this floor near the north wall is located the heater of the steam-heating plant. The temperature is maintained at about 60° F. throughout the winter, but in the immediate vicinity of the heater the temperature is, of course, considerably higher. From time to time cabbages were brought in from the storage banks and piled on the floor between the heater and the wall until used for feeding. A certain amount of decay always set in, especially in some of the outer leaves of the cabbages which had previously been slightly frozen. The

warmth from the heater and the odor of decaying cabbage proved very attractive to house flies, and large numbers congregated here early in the fall and were present in considerable numbers throughout the winter. That the house flies present here during March and the early part of April, 1917, were not the survivors but rather the offspring of those which had congregated here in the fall of 1916 is quite clearly indicated from the following observations:

It was noted that large numbers of these flies were carried off during December and early January by the attacks of a fungus, doubtless *Empusa muscae*. For example, on January 4, on one of the supporting posts near the heater, 68 victims of the fungus were counted. These were swept off, and six days later about 30 fresh victims were counted. Shortly after this date all the woodwork of the interior of the building was painted and no more deaths from fungus were noted, but dead flies were occasionally found on the floor below windows or between the windows and screens. In spite of these deaths there was no very noticeable falling off in the number of active adults which frequented the pile of cabbages. It was evident that breeding was taking place and that freshly emerged flies were steadily appearing to take the place of those killed by natural causes.

The breeding places were not definitely located. The cages for rabbits and guinea pigs were kept in excellent condition. All excrement, soiled litter, and waste food were removed from the cages every day, or at least every other day, and hauled away from the building. Floors under and around the cages were swept clean. The only possible place for the development of the larvæ seemed to be in the decaying portions of the cabbage. No lot of cabbage remained on the floor near the heater more than four or five days before it was used for feeding. It is possible, however, that flies may have oviposited on some decaying portion of the cabbage, and the issuing larvæ may have had three or four days development before being disturbed. Then, some may have been transferred with the food to the animal cages, where they possibly would have had time to complete their growth and to pupate in some obscure corner of the cage or in clean litter, and thus escape the cleaning process. The warmth near the heater and the warmth in the cages from the bodies of the animals would be sufficient to carry through development to pupation at about the normal rate. A study of the adults showed conclusively, however, that breeding was taking place and that freshly emerged flies were appearing from time to time.

Deposition of eggs was easily induced when a suitable medium was exposed. Thus, on December 13 a 1-gallon, wide-mouthed glass jar was half filled with moistened bran and exposed on a low support near the pile of cabbages. The warmth soon produced an active fermentation and eggs were obtained on December 15. Again, similar depositions were obtained on December 22, December 28, January 6, and other sub-

sequent exposures. In no case was there failure to obtain eggs within two or three days after exposure of the moist bran. The eggs so obtained were, of course, removed from the building in order not to interfere with the normal course of events.

The foregoing observations on deposition were supplemented by a study of a number of females captured from time to time during the winter. They were first examined to determine whether or not they were freshly emerged. This is done by exerting a little pressure on the sides of the thorax with a pair of forceps. In the case of very young flies this causes the ptilinum to extrude, even if it had previously been completely retracted. In the course of some other experiments it was found that in flies exposed to rather low temperatures the ptilinum may retain this elasticity for as long as 12 days, but at the temperature of this animal house it is probable that the ptilinum can not be forced out after the flies are three or four days old. When such pressure does not cause the ptilinum to extrude, it is certain that the fly is past this early stage of plasticity, but how much older it may be is, of course, unknown.

After this examination the flies were dissected and the spermathecæ were examined for the presence or absence of spermatozoa, and the size and development of the ovaries was studied. A regular procedure was adopted in this study, as follows: The abdomen was removed from the freshly killed fly, and from this the entire reproductive system was removed. This was put on a hollow ground-glass slide and covered with physiological salt solution. The spermathecæ were then removed and mounted on a plain glass slide in salt solution and examined under the compound microscope (4-mm. objective). A little pressure on the cover glass will cause the chitinous capsule to break open and the presence or absence of spermatozoa is easily determined. Plate 7, figure G, gives an idea of the appearance of the spermathecæ just after they have been thus broken open. The spermatozoa in lively wriggling masses are seen issuing from the cracks of the capsule walls and also from the severed ends of the ducts. The lines of the figure which represent the spermatozoa are relatively darker and heavier than they actually appear under the microscope.

The ovaries were fixed in Carnoy's fluid and washed in alcohol and then stained in carmalum or borax carmine. After destaining and dehydrating, the ovarioles were separated by careful manipulation with needles and mounted in balsam. Sometimes a little picric acid was added to the 95 per cent alcohol during the dehydrating process in order to bring out the chitinous structures more clearly. The ovaries were found in all stages of development. Camera-lucida drawings of certain well-marked stages were made. These are shown in Plate 7, figures A to F. They are drawn to the same scale, the No. 10 eyepiece and the 16-mm. objective being used. Most of the figures show the structures in optical section, but the surface markings have been represented

on some of the larger cells. These six stages were selected arbitrarily as standards of reference, and all the other ovaries were compared with them and grouped according to the stage which they most closely approached. The results of this study are given in Table III.

TABLE III.—*Showing presence of spermatozoa and the stage of development of the ovaries in house flies taken during the winter in a warm building, Bethesda, Md., 1917*

Date.	Number of females dissected.	Condition of spermathecæ.		Ptilinum extruded (freshly emerged flies).	Condition of ovarioles at developmental stages 1–6.					
		Spermatozoa present.	Spermatozoa absent.		1	2	3	4	5	6
1917.										
January 10	6	4	2	.....	[a] 1	[a] 1	2	.....	2	.....
January 15	2	2	.....	.....	.....	.....	.....	1	1	.....
January 17	4	4	.....	.....	.....	.....	2	1	1	.....
January 24	2	1	1	.....	.....	[a] 2	.....	.....	.....	.....
January 30	2	1	1	1	ap.e. 1	.....	.....	.....	.....	1
February 2	4	3	1	.....	.....	[a] 1	1	.....	1	[b] 1
February 6	4	4	.....	.....	.....	.....	1	1	2	.....
February 8	4	2	2	.....	[a] 2	1	.....	.....	1	.....
February 12	3	2	1	2	ap.e. 1	p.e. 1	1	.....	.....	.....
February 13	3	3	.....	.....	.....	1	2	.....	.....	.....
February 16	4	4	.....	.....	.....	.....	3	.....	1	.....
February 17	2	2	.....	.....	.....	.....	1	.....	1	.....
February 21	4	2	2	1	ap.e. 1	[a] 1	.....	.....	1	1
February 26	2	1	1	.....	.....	[a] 1	.....	.....	.....	[c] 1
March 9	4	3	1	.....	.....	1	.....	[a] 2	.....	[c] 1
March 13	3	2	1	.....	.....	[a] 1	2	.....	.....	.....
March 17	4	4	.....	.....	.....	.....	2	.....	2	.....
March 21	2	2	.....	.....	.....	.....	1	1	.....	.....
Total	59	46	13	4	6	11	18	6	13	5
Percentage of total	.....	78	22	6.7	10.1	18.6	30.5	10.1	22	8.4

[a] Indicates ovaries of flies from which spermatozoa were absent.
p.e. Indicates those in which ptilinum could be extruded.
[b] Two eggs lodged in oviduct showing that deposition had recently occurred.
[c] Mature egg lodged in oviduct, showing that deposition had recently occurred.

Table III shows that of the 59 females examined during the period from January 10 to March 21, 1917, 46, or 78 per cent, contained living spermatozoa in the spermathecæ, while in 13, or 22 per cent, the spermathecæ were empty. In 6 flies, or 10 per cent, the ovaries were in the first stage of development. Correlated with this was the entire absence of spermatozoa and the fact that in 3 of them a slight pressure on the thorax caused the ptilinum to extrude. This proves conclusively that they were very young flies. Of the 11 flies, or 18.6 per cent, with ovaries in the second stage of development, spermatozoa were found in only 3 cases, and the ptilinum could be extruded in 1 case. They were but slightly older than those of stage 1. With one exception (a fly with

ovaries in the fourth stage) all flies with ovaries past the second stage of development contained spermatozoa. In 13 flies, or 22 per cent, the ovaries were apparently mature, and eggs were ready for deposition. In 5 flies, or 8.4 per cent, the condition of the ovaries indicated clearly that deposition had occurred only a short time previous to the examination. The ovarioles had the appearance of figure F (Pl. 7), and in 3 cases mature eggs were seen lodged in the oviduct at the point where the oviducts unite to form the common oviduct.

A comparison of the foregoing findings with the conditions found in *Pollenia rudis* is very instructive. There is hardly any doubt that Pollenia hibernates in the adult state. It is found at all times throughout the winter in attics, unoccupied rooms, and the like, and is often seen outdoors in mild weather. Frequent collections were made during the winter of 1916–17, and the spermathecæ and ovaries were studied in the same way as those of the house fly. The spermathecæ were found to be empty in all cases examined during December, January, and February. The first spermatozoa were found in a female examined on March 22. Correlated with this was the fact that, without exception, all ovaries corresponded in development to stage 1 (Pl. 7, A) of the house fly. Not until early in March was any more advanced stage of development encountered. This agrees exactly with Kisliuk's observations (*13*) on Pollenia at Columbus, Ohio, where he found no development of the ovaries until early in March. Kisliuk (*13*) also succeeded in obtaining eggs from specimens of Pollenia captured on March 21. A specimen of Pollenia was kept alive in a cage in the screened insectary at Bethesda, Md., for a period of about four months during the winter, and during this time the temperature reached a minimum of −1° F. It appears, then, that those individuals of Pollenia emerging in October and November overwinter as adults, being active on warm days, but clustering in corners and hiding in cracks and crevices during cold periods. The ovaries remain undeveloped until spring. During warm periods in March the ovaries begin to develop, copulation takes place, and eggs are soon ready for deposition.

Now, if *Musca domestica* did hibernate as an adult, one would expect to find a similar suspension of sexual development even in warm buildings, and to find the reproductive power conserved to be exercised only in the spring when outdoor activities could be resumed. *M. domestica*, however, does not show any such retardation of ovarian development; nor does it display the same ability to withstand the effects of cold. Another important fact is that *M. domestica* was never collected during the winter in attics and other unused but protected places where Pollenia is found in such abundance, although experiments with caged house flies showed that the temperature of such locations was most favorable for the prolongation of life. In other words, adults of *M. domestica* were found during the winter only where food was available, and persisted

throughout the winter only where conditions permitted breeding to continue uninterruptedly.

Jepson (*12*) and other observers have proved experimentally that house flies will breed during the winter under the proper conditions. The foregoing studies on the development of the ovaries and the behavior of the flies in the animal house at the Bethesda station, and Kisliuk's observations (*13*) in the animal house at the Ohio State University and in the Columbus (Ohio) garbage disposal plant, prove that breeding does normally take place in locations where artificial heat, food material, and breeding media are available. Breeding continues all winter in such places, and then on warm days in the spring the flies escape through open windows or doors and take up their usual outdoor activities. This is very probably the explanation of the early appearance of those flies taken in traps on the Bethesda farm in March, 1917, and also of those found by Dr. Henry Skinner (*17*) on March 3, 1913, since in a city there are doubtless many places where breeding occurs throughout the winter.

RELATION OF TEMPERATURE TO ACTIVITY

The behavior of house flies at ordinary summer temperatures has been treated fully in the works of L. O. Howard (*11*), Hewitt (*8*, *9*), Graham-Smith (*7*), and others. But the following brief notes on the effect of low temperatures on activity have a direct bearing on the question of overwintering. Observations were made on both laboratory-reared caged flies and wild flies:

At temperature of

- 65° F. . Flies are quite active.
- 55° F. . Flies are fairly active. Copulation was noted at this temperature, but no sexual activity has been noted at lower points.
- 52° F. . Flies only slightly active.
- 42° F. . Flies move rather weakly when disturbed, but are otherwise inactive. In cages they are usually found on the sides or in corners near the top.
- 40° F. . Flies inactive; crawl feebly when touched.
- 34° F. . Flies inactive; may move legs in weak uncoordinated way when touched.
- 30° F. . One male survived exposure for one week at this temperature. (*10*).
- 29° F. . Usually no reaction when touched, but they quickly revive when brought into a warm room.
- 25° F. . Appear to be dead, but most of them revive when brought into a warm room.
- 22° F. . Apparently dead, but in some cage experiments it was found that a considerable percentage revived when warmed after exposure to this minimum during the preceding night.
- 12° F. . All dead; no recovery when taken to a warm room. "None survived exposure for one week." (*10*).
- 10° F. . All dead.

The fatal temperature is thus shown to be quite low, and the fact that many revive after exposure to temperatures as low as 22° F. explains why flies persist outdoors in this latitude as late as the first week in December. The most important point is that flies will resume activities when the temperature is raised, even after exposure to rather low temperatures. There is no hibernation in the sense of long-continued suspension of activity during the winter months. Dove (5) states that he has never—

> observed living adults to remain quiescent for more than a few days. The natural heat of the sun or very slight artificial heat will cause them to become active.

OVERWINTERING OF LARVA AND PUPA STAGES

Probably every observer who has planned any work on this problem has started out with the assumption that "it might reasonably be thought that the pupæ would prove more resistant than the earlier stages" (3), and there have, in fact, been many attempts to carry the puparia of the house fly through the winter. "But," says Copeman, "so far as I am aware, no observer has succeeded in breeding out the flies from pupæ kept through an average winter in this country" [England]. "Nor," according to Hewitt's statement (9), "has it ever been possible in my breeding experiments in Canada and in England to carry the insect through the winter in the pupal state." Lyon (14) reports some experiments started on October 19, 1914, at Boston, Mass. Thirty-seven lots, of 100 puparia each, were buried in wet and dry sand, wet and dry loam, wet and dry horse manure, and leaf mold. These materials, with pupæ, were placed in glass jars, some in the basement of a stone building, some in an unused greenhouse, some in sheltered positions outdoors, and some in exposed positions outdoors. No flies emerged from those lots placed outdoors. Several hundred emerged from those kept in the greenhouse and in the basement, but even from these none emerged after December 1, 1914. Upon examination on June 23, 1915, the pupæ appeared normal, but on being broken open they were found to have completely dried out.

The writer has tried similar experiments and obtained similar results. About 1½ quarts of pig-manure-bran mixture containing approximately 900 puparia were collected at Bethesda, Md., on November 14, 1914. This mass was put in a large wooden box, covered with 3 or 4 inches of sand, and the box, covered with a plate of glass, was placed on a shelf of a screened insectary. House flies in considerable numbers emerged on mild days up to December 4. None emerged after that date. On March 17, 1915, the box was transferred to a warm room in a greenhouse and remained there until April 1, but no flies appeared. Another collection of pupæ made on December 3, 1914, and handled in the same way, also showed no emergence after December 4. In a third experiment special precautions were taken to prevent freezing, and pupæ of known

age were used. From rearing experiments in the greenhouse eggs were obtained January 9, 1915. These hatched and were carried through in horse manure confined in a large wooden box. From this lot about 150 puparia were collected on January 26, immediately placed in a box (8 by 8 by 10 inches), and covered with 4 inches of dry sand. The box was taken to the apicultural laboratory at Drummond, Md., and buried to within 1 inch of the top of the box in the ground near the east wall of the building. With the help of Mr. G. S. Demuth thermocouples were inserted in the sand among the puparia. The box was then covered with a glass plate and over this was placed a square of rubberized roofing and a large quantity of sawdust. Temperature records were taken eight times daily from this date until April 26, by means of the electrical apparatus used by Phillips and Demuth (*16*) in their work on the temperature of the bee cluster in winter. The actual temperature to which the puparia were exposed was uniformly low, but never less than 34° F. There was a gradual rise in temperature until 45° F. was reached, on April 26, when the readings were discontinued. On May 17 the box was taken from the ground. No flies had emerged. The puparia were collected and examined. While their external appearance was normal, of 10 puparia which were dissected, 7 were found to contain a brown sticky liquid, and in 3 the pupæ, although well formed, were completely dried up. The other puparia were kept in ordinary garden soil until July 17, but no flies emerged.

All these experiments are open to the criticism that the conditions were more or less artificial, and that relatively small numbers of puparia were used. Attempts to provide more nearly natural conditions and to determine the possibility of overwintering in large heaps of manure were first reported by Bishop, Dove, and Parman (*2*). They report two cases in which they succeeded in carrying the house fly through the winter in the immature stages. In one of these, at Dallas, Tex., 3 barrels of horse manure, cow manure, and straw were put in a cage on November 26, 1913. No flies emerged after December 27 until April 16, 1914, when 4 specimens appeared. Others emerged May 26. Larvæ were found in some numbers up to March 21. Thus it was shown that the house fly lived in the larva stage for a period of 115 days, and in the larva and pupa stages until April 16 and May 26, periods of 141 to 181 days. In a second experiment, at Uvalde, Tex., horse manure and straw were put in a cage on December 6, 8, 9, and 13, 1913. Emergence continued during warm periods throughout the winter. From 1 to 9 flies emerged daily from March 1 to 18, and on each of three days (Apr. 1, 2, and 4) a single fly emerged. Dove (*5*) gives further details of these experiments.

The publication of the experiments of Bishopp, Dove, and Parman (*2*) brought up the question whether they were paralleled under more severe winter conditions, or whether they obtained only in the moderate climate of Texas and the Gulf coast. Experiments similar to these were carried

out by the writer at Bethesda, Md., during the two winters of 1915–16 and 1916–17, but not until the winter of 1916–17 was any evidence obtained which tended to show that overwintering in the larva and pupa stages was possible in the more northerly latitudes. The results of these experiments are here briefly reviewed.

Six wooden frames 7 feet square and 2 feet high were set in the ground to a depth of 6 inches. On these were placed pyramidal screened covers. The pyramids were slightly truncated and provided with a 6-inch opening at the top, over which a large flytrap was fastened. The whole was carefully fitted, so that there was no escape for flies except into the trap at the top. Migrating larvæ would have to burrow at least 6 inches into the soil in order to escape under the wooden sides of the cages. From October 22 to 29, 1915, 8 bushels of fresh horse manure and straw were put in each box. To this was added about 5 quarts of pig-manure-bran mixture, which had been collected from a heap near some pigpens and which contained thousands of larvæ in different stages. Additions of both horse manure and pig manure were made at intervals until December 8, 1915, when there was a total of about 25 bushels. The pyramidal covers, with flytraps attached, were put in place on November 4, and records of emergence from three cages were kept during the fall and of all six cages during the following spring.

From the three cages records of which were kept during the fall emergence ceased after December 3, 1915. The number and species are given in Table IV.

TABLE IV.—*Number and species of flies emerged from three heaps of horse and pig manure from November 4 to December 3, 1915*

Species.	Total number of flies emerged.	Species.	Total number of flies emerged.
Musca domestica	1,062	*Muscina assimilis*	1
Stomoxys calcitrans Linnaeus	242	*Orthellia cornicina*	1
Morellia micans Macquart	2	*Phormia regina*	1

During the following spring emergence began April 6 and continued as late as June 1, 1916. The records for all six heaps are given in Table V.

In these experiments *Musca domestica* was found emerging up to December 3, 1915, but none were taken after this date, although the heaps were kept under observation until June 1, 1916. The same is true of *Stomoxys calcitrans*. Of the six species taken during the fall only one (*Muscina assimilis*) appeared again in the spring in these traps. Eight species in addition to the three or four species of the Sarcophagidae were taken during the spring, and thus proved to have overwintered in the larva and pupa stages. Of these *Hydrotaea houghi* and *Ophyra leucostoma*

are not normally found in horse manure, but were doubtless introduced in the pig-manure-bran mixture.

TABLE V.—*Number and species of flies emerged during the spring of 1916 from six heaps of overwintered horse and pig manure*

Species.	Total number of flies emerged.	Period of emergence.
Muscina assimilis	8	May 4 to 20.
Muscina stabulans	1	May 4.
Fannia canicularis Linnaeus	35	April 6 to May 22.
Hydrotaea houghi Malloch	237	April 24 to May 31.
Ophyra leucostoma Wiedemann	594	April 28 to May 31.
Phorbia sp	4	April 28 to May 4.
Scatophaga furcata Say	26	April 6 to May 6.
Syritta pipiens Linnaeus	3	May 6 to 22.
Sarcophagidae	18	May 2 to 29.

Three experiments were carried out during the winter of 1916–17. They were started on October 3, 1916, by placing in each of three wooden frames 8 bushels of fresh horse manure containing little straw and seen to be quite heavily infested with larvæ of both *Musca domestica* and *Stomoxys calcitrans*. Additions of horse manure were made from time to time up to January 8, 1917, when each heap contained 40 bushels of manure. Larvæ of *Musca domestica* and *Stomoxys calcitrans* were seen in all manure added to the heaps up to and including that of October 24, all additions after this date appearing to be free from infestation.

The first of these three experiments was managed somewhat differently from the others. On October 9, 1916, 1 quart of moist bran containing hundreds of house-fly larvæ was put on the heap. Again on three occasions—December 27, 1916, and January 4 and 10, 1917—2 quarts of moist bran which had been previously exposed in the animal house and was heavily infested with fly larvæ were buried in the heap. In this way fully 2,500 house-fly larvæ were added to the normal infestation of the manure. The pyramidal cover, with attached flytrap, was put in place on November 13. From this date to December 11 the following species were taken from the trap:

Musca domestica	20	*Fannia canicularis*	1
Stomoxys calcitrans	247	*Scatophaga stercoraria*	2

No further emergence was noted during the winter until March 22, 1917. The trap was regularly emptied from that time until June 4, the catches being summed up in Table VI.

TABLE VI.—*Total number of flies emerged during the spring of 1917 from one heap of horse manure to which infested bran was added*

Species.	Total number of flies emerged.	Period of emergence.
Musca domestica	1	April 30.
Muscina assimilis	1	April 16 to 23.
Muscina stabulans	1	May 14 to 21.
Ophyra leucostoma	18	April 9 to 30.
Hydrotaea armipes Fallén	36	April 9 to May 14.
Fannia canicularis	39	April 16 to May 21.
Other species of Anthomyiidae	22	April 16 to 30.
Scatophaga stercoraria	26	March 22 to April 30.
Scatophaga furcata	145	April 9 to May 14.

The single specimen of *Musca domestica* taken from the trap on April 30 was plainly freshly emerged, having bright colors, soft body, and ptilinum not fully retracted.

Two other experiments were conducted in the same way as the one just described except that no bran with larvæ was added at any time, and therefore no larvæ were introduced after October 24. The manure added after this date was not infested. From November 13 to December 11, 1916, the following flies emerged from these heaps:

Musca domestica	34
Stomoxys calcitrans	200
Scatophaga stercoraria	1

No emergence took place from December 11 to April 2. From April 2 to June 4, a total of 347 flies were taken, as shown in Table VII.

TABLE VII.—*Total number of flies emerged during the spring of 1917 from two heaps of over-wintered horse manure*

Species.	Total number of flies emerged.	Period of emergence.
Musca domestica	1	April 23.
Stomoxys calcitrans	1	May 14.
Myospila meditabunda	3	April 20 to May 14.
Muscina assimilis	2	April 30 to June 1.
Fannia canicularis	33	April 23 to May 21.
Hydrotaea armipes	76	April 23 to May 25.
Other species of *Anthomyiidae*	50	April 16 to May 14.
Scatophaga furcata	158	April 2 to May 14.
Scatophaga stercoraria	23	April 2 to May 21.
Total	347	

From these three experiments only two specimens of house flies emerged during the spring. If this number is too small to prove this to

be the usual method of overwintering, at least it proves the possibility in the latitude of Washington. From a mass of pupæ collected in a pile of rabbit and guinea-pig manure on February 26, Kisliuk (*13*) was successful in rearing four house flies on March 10 and 12. McDowell and Eastwood (*15*) record observations of the same kind. Whether the house fly can overwinter in the larva and pupa stages in more severe climates is still doubtful. C. W. Howard (*10*) concludes from his observations that—

> the temperature of Minnesota winters is not favorable to the overwintering of the house fly in any except the adult stage and that stage only in places where there is a sufficiently high temperature and where food conditions are favorable.

SUMMARY AND CONCLUSIONS.

The present status of our knowledge of the overwintering habits of the house fly seems to the writer to warrant the following conclusions:

In the latitude of Washington, D. C., the house fly may overwinter in two ways: (1) By continued breeding in warm places where food and media for deposition are available, and (2) in the larva and pupa stages in or under large manure heaps.

There is no evidence whatever to show that house flies do or can persist as adults from November to April, either outdoors, in protected stables, or in attics or heated buildings. Temperatures of 12° or 15° F. are quickly fatal, and there is every reason to believe that any temperature below freezing is fatal if continued long enough. In heated buildings their life is not prolonged beyond that of summer at like temperature, nor is there any suspension or retardation of sexual development or activity.

It is known that house flies continue to emerge from manure heaps as late as the first week in December. Many of these late forms will find their way on mild days to heated buildings, and those which do not are quickly killed. Those that find their way to heated buildings are attracted, as in summer, to odors of food and will congregate in kitchens, dining rooms, restaurants, bakeries, animal houses, and the like. If no food is at hand they will quickly perish. When food is available they may continue alive through December and January and even into February, if not destroyed by fungus attacks. But there are neither experiments nor observations to show that they can continue throughout the winter until temperatures are again favorable for outdoor activity and egg laying. If flies find access in the autumn to heated buildings, where both food and media for deposition are available, such as animal houses or restaurants in which sufficient attention is not given to the disposal of garbage or kitchen wastes, they will continue breeding throughout the winter. In such cases the flies present in March and April are the offspring, not the survivors, of those which found their way to such places the preceding autumn. It is probable that this method of over-

wintering is much more widespread than is now realized, especially in cities where there must be several foci from which flies escaping on warm days in March and April, survive to produce the hordes that begin to appear late in May.

The possibility of house flies overwintering in the larva and pupa stages has been demonstrated at Washington, D. C., and at Columbus, Ohio, as well as for the milder regions of Texas. But whether this method of overwintering in these stages or by continued breeding is the more common or more successful can not now be stated. To judge from experiments with larvæ and pupæ and from the fact that house flies do not appear in large numbers until late in May or early in June it would seem that only a very small percentage of larvæ, which are present in manure heaps in the autumn, live through the winter and give rise to the adults in the spring.

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PLATE 13

Musca domestica:

A–F.—Various stages in the development of the ovarioles from the time of emergence of the fly until after deposition of eggs. Camera-lucida drawings from ovarioles stained in carmalum or borax carmine. All drawn on same scale. No. 10 eyepiece and 16–mm. objective.

G.—Two spermathecæ. Camera-lucida drawing from fresh preparation showing spermatozoa escaping from the severed ends of the ducts and from the splits in the sides of the capsules caused by pressure on the cover glass.

A
B
C
D
E
F
G

SOIL ACIDITY AS INFLUENCED BY GREEN MANURES

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INTRODUCTION

The study of the general fertilizer series of plots at the Pennsylvania Experiment Station, which have been continuously treated according to different systems of fertilizing ever since 1881, has shown some instances of large lime requirements, particularly in the case of the soils of plots 30, 31, and 32, treated, respectively, with a complete fertilizer containing different quantities of nitrogen supplied in the form of ammonium sulphate. Experiments reported in recent years have shown clearly by the ordinary tests, that these soils are distinctly acid, that the common rotation crops fall off in yields in proportion to the degree of acidity of these soils, and that the use of either lime or finely ground limestone in quantity sufficient to make the lands neutral is all that is necessary to bring them back to normal bearing.

One of the questions raised by the facts just stated concerns the cause or causes producing the sour or lime-deficient conditions here observed. Among these causes commonly regarded as operative to set up an acid condition, or one of pronounced lime requirement in a soil, is the accumulation in it of a large amount of organic matter decaying under conditions or by the action of agents that form acid materials from it. On all cultivated soils, sods and crop residues are customarily plowed under and might serve as the raw material for the production of acid substances and, indeed, may have at the outset a direct acid effect, because they themselves contain, especially in the immature condition, considerable quantities of acid-acting constituents.

Doubtless, however, the heavy cover crops and intercrops, turned under as green manures, should most strikingly show the kinds of effects here in question. Practical experience has sometimes shown temporary ill effects to follow the turning under of especially rank growths of green manures, and their stage of maturity and their kind are regarded as having something to do with the appearance or nonappearance of these ill effects. This particular phase of the green manure subject has not to our knowledge, been elsewhere studied.

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MANURES USED

In our study of organic crop residue as possibly contributory to the development of acidity in these ammonium-sulphate plots we have used, as the organic materials to be mixed with the soil, various green-manuring plants, leguminous and nonleguminous, and some common weeds and less desirable grasses. These have been used both fresh and air dried with the thought that in the latter condition, while the plant materials would not have the exact balance of composition possessed by the corresponding mature growths, they would at least resemble the mature plants in physical quality and resistance to the bacterial penetrations. To the list of organic materials used (Table I), barnyard and poultry manures were added to ascertain what differences of behavior they might develop, with the view of thus more sharply distinguishing the real factors.

TABLE I.—*Green manures used in experiments*

Series I (applied green).	Series II (applied air-dried).
Legumes:	Legumes:
Soy bean (*Soja max*).	Soybean.
Canada field pea (*Pisum sativum*).	Sweet clover.
Sweet clover (*Melilotus alba*).	Alfalfa.
Alfalfa (*Medicago sativa*).	Red clover.
Red clover (*Trifolium pratense*).	Hairy vetch.
Hairy vetch (*Vicia villosa*).	
Nonlegumes:	Nonlegumes:
Wheat (*Triticum* spp.).	Rape.
Rape (*Brassica napus*).	Corn.
Oats (*Avena sativa*).	Rye.
Corn (*Zea mays*).	Redtop.
Rye (*Secale cereale*).	Sorrel.
Timothy (*Phleum pratense*).	
Redtop (*Agrostis alba*).	
Sorrel (*Rumex Acetosella*).	

The wheat, rape, and soy beans used were young, tender plants. The wheat was obtained from a pot experiment, where the growth was about 1 foot high. The other materials were removed from the field on July 6, and they represented an average stage of maturity at that period.

The composition of the several manures as used in Series I, with respect to the point judged to be most important in the present study, is given in Table II.

TABLE II.—*Composition of green manures and others used in experiments*

Material.	Original moisture content.	In water-free substances.					Quantity of fresh material to yield 10 gm. of dry matter.
		Organic matter.	Ash.	Alkalinity of ash.[a]	Water-soluble acidity of manures.	Nitrogen.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*Gm.*
Legumes:							
Soy bean	76. 7	78. 04	21. 96	6. 92	1. 70	2. 78	42. 9
Canada field pea	80. 7	88. 85	11. 15	7. 80	4. 02	3. 65	50. 5
Sweet clover	72. 5	92. 16	7. 84	5. 27	2. 90	3. 12	36. 3
Alfalfa	65. 8	91. 37	8. 63	7. 82	2. 13	2. 76	29. 2
Red clover	61. 6	92. 75	7. 25	5. 63	. 28	1. 94	26. 0
Hairy vetch	66. 4	93. 04	6. 96	3. 27	1. 91	2. 84	29. 8
Nonlegumes:							
Wheat	75. 2	89. 36	10. 64	6. 00	. 19	3. 56	40. 2
Rape	89. 1	80. 33	19. 67	10. 48	2. 88	4. 79	91. 7
Oats	83. 2	88. 46	11. 54	5. 35	8. 15	3. 08	59. 5
Corn	87. 9	91. 29	8. 71	4. 70	5. 92	2. 72	91. 7
Rye	56. 1	95. 40	4. 60	1. 27	3. 07	1. 33	22. 7
Timothy	60. 1	93. 76	6. 24	2. 32	. 23	1. 29	25. 0
Redtop	60. 2	93. 99	6. 01	1. 60	. 42	1. 07	25. 1
Sorrel	80. 5	90. 18	9. 82	3. 67	3. 01	1. 98	50. 2
Other manures:							
Poultry manure	64. 7	71. 38	28. 62	9. 65	(b)	3. 35	28. 3
Barnyard manure	74. 7	60. 83	39. 17	5. 52	(b)	1. 57	39. 5

[a] In terms of the calcium-carbonate equivalent. [b] Alkaline.

SOIL USED

With the view of detecting a change in the degree of acidity or lime requirement after the addition of the green manures, a soil already distinctly acid was chosen. This soil was taken from plot 32. The lime requirement of this soil, as shown by concordant duplicate determinations by the Veitch method, was equivalent to 4,644 pounds of calcium carbonate to the acre 7 inches (2,000,000 pounds). This quantity will hereafter in this paper be called the "limestone requirement." The soil employed had, before this determination, been freed from stones and roots by sifting through a screen with meshes of a diameter of 3 mm. Before use it was thoroughly mixed to make it uniform throughout.

PREPARATION OF GREEN MANURES AND SOIL MIXTURES

The fresh green manures were cut fine with scissors and then passed through a sausage mill until thoroughly subdivided. The water removed from the green residues during the process was carefully replaced. The fine product was used for the experiment of Series I. Other portions of the same materials were carefully air-dried and similarly subdivided for use in Series II.

Of the fresh materials thus prepared, the respective quantities stated in the last column of Table II as equivalent to 10 gm. of dry matter were

each thoroughly mixed with 500-gm. portions of the soil. The mixture thus represents an addition of 20 tons of green manurial dry matter to 2,000,000 pounds, or an acre 7-inch layer of soil.

Like mixtures of corresponding amounts of the air-dried manurial substances were used with 500-gm. portions of the soil from Series II.

SUBSEQUENT TREATMENT

The mixtures thus prepared were kept in large jars freely exposed to the air and sunshine for two months; thereafter, until the close of the experiment, in a well-ventilated room. The entire period extended from July 7, 1914, to April 7, 1915. The jars were weighed at frequent intervals, and the losses of weight made up by the addition of distilled water so as to keep the soil quite close to the optimum moisture content with which it was started, in order that, as respects the moisture present, the fermentative conditions might be more favorable.

A control sample of the original untreated soil was similarly kept during this period.

LIMESTONE-REQUIREMENT DETERMINATIONS

At periodic intervals the limestone requirements of these mixtures were determined. To obtain samples for this purpose, the entire mass of material was removed from the jar, thoroughly mixed, a sample sufficient for duplicate determinations withdrawn, and the remainder of the soil returned to its jar, and put as nearly as possible into the original condition of compactness. The determinations were made by the Veitch method, the same as for the original soil.

Unfortunately the determinations were not begun until two weeks after the mixtures were prepared and exposed in moist condition as above described. The changes in limestone requirement during this period of fermentation may, however, be approximated if we assume that in each case it corresponds to the sum of the limestone requirement of the soil and the calcium-carbonate equivalent of the water-soluble acids in the manurial material used—that is, the sum of the limestone requirement of the soil and the calcium carbonate equivalent of the water-soluble acids in 40,000 pounds of the manurial dry matter, the manurial requirement for 2,000,000 pounds of soil.

The sum thus obtained must be reduced to the equivalent for 2,000,000 pounds of the mixture, because all subsequent determinations are based upon that quantity of the mixtures.

If the colloidal substances in the plant materials are capable of absorbing lime from limewater so as to increase the apparent acidity of the materials, the theoretical limestone requirement thus calculated is, of course, too low.

SERIES I: FRESH GREEN MANURES

For each mixture of the soil with the fresh material (Series I) this theoretical original limestone requirement and the actual requirement determined at the several periods of the experiment are stated in Table III; the amounts and direction of change during the corresponding intervals, in Table IV.

The figures of Table IV show that all the mixtures exhibited very great differences in limestone requirement from time to time during the nine months through which these observations extended. The degrees of variation significant of real differences, as distinguished from such as are due to difficulties of sampling and analysis, are well established by comparison of the duplicate determinations made at each period and for each mixture from separate portions of the mixture or soil itself. The average difference between such duplicates corresponded to about 200 pounds of limestone per acre.

TABLE III.—*Limestone requirement of green manures (Series I)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	Original (theoretical).	Period 1 (2 weeks).	Period 2 (4 weeks).	Period 3 (3 months).	Period 4 (5 months).	Period 5 (7 months).	Period 6 (9 months).
Control soil	4,644	4,644	4,711	4,644	4,677	4,871	4,904
Legume mixtures:							
Soy bean	5,220	3,627	4,712	4,678	5,661	5,994	5,650
Canada field pea	6,136	3,593	4,104	4,238	5,865	6,163	5,960
Sweet clover	5,690	3,187	4,949	4,983	6,509	6,570	6,502
Alfalfa	5,388	2,915	4,136	3,695	5,153	5,210	4,847
Red clover	4,663	713	1,153	.....	4,848	5,040	4,135
Hairy vetch	5,302	2,882	4,949	4,305	5,865	6,062	4,542
Subaverage	5,400	2,819	4,000	3,650	5,650	5,840	5,273
Nonlegume mixtures:							
Wheat	4,627	3,492	4,644	5,153	7,594	7,493	7,182
Rape	5,682	4,305	4,949	5,017	6,068	6,366	6,298
Oats	7,749	3,525	5,051	5,152	6,068	5,892	6,163
Corn	6,874	3,932	5,457	5,525	6,544	7,182	6,876
Rye	5,757	3,254	4,543	3,831	4,958	4,813	4,508
Timothy	4,643	2,610	4,271	3,831	5,356	5,243	4,847
Redtop	4,718	2,848	4,983	3,288	4,712	5,790	4,474
Sorrel	5,733	1,729	2,949	2,237	3,797	4,813	4,101
Subaverage	5,723	3,212	4,606	4,254	5,637	5,949	5,556
Barnyard-manure mixture	(?)	3,187	3,865	3,797	4,678	5,549	4,575
Grand average	5,584	3,053	4,314	3,982	5,578	5,879	5,377

TABLE IV.—*Changes in the limestone requirement from period to period (Series I)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	Period 1 (first 2 weeks).	Period 2 (second 2 weeks).	Period 3 (second and third months).	Period 4 (fourth and fifth months).	Period 5 (sixth and seventh months).	Period 6 (eighth and ninth months).	Total in 9 months.
Control soil	0	67	−67	33	194	33	260
Legume mixtures:							
Soy bean	−1,593	1,085	−34	983	333	−344	450
Canada field pea	−2,543	511	134	1,627	298	−203	−176
Sweet clover	−2,503	1,762	34	1,526	61	−68	842
Alfalfa	−2,473	1,221	−441	1,458	57	−363	−541
Red clover	−3,950	440	−1,154	?4,848	192	−905	−528
Hairy vetch	−2,420	2,067	−645	1,560	197	−1,520	−760
Subaverage	−2,580	1,181	−351	2,000	190	−567	−119
Nonlegume mixtures:							
Wheat	−1,135	1,152	509	2,441	−101	−311	2,555
Rape	−1,377	644	68	1,051	298	−68	616
Oats	−4,224	1,526	101	916	−176	271	−1,586
Corn	−2,942	1,525	68	1,019	638	−306	2
Rye	−2,503	1,289	−712	1,127	−145	−305	−1,249
Timothy	−2,033	1,661	−440	1,525	−113	−396	204
Redtop	−1,870	2,135	−1,695	1,424	1,078	−1,316	−244
Sorrel	−4,004	1,220	−712	1,560	1,016	−712	−1,632
Subaverage	−2,511	1,394	−352	1,383	312	−393	−167
Barnyard-manure mixtures	?−1,457	678	−68	881	871	−974	? −69
Grand average	−2,468	1,261	−215	1,596	300	−501	−141

The strength of the limewater reagent used was carefully standardized at each period; and even had it not been, the great differences in limestone requirement of the manurial mixtures, as compared with the control soil at the successive times of examinations, would bar such reagent variations as an explanation of the facts here recorded.

It is strikingly shown that the direction in change of limestone requirement was not continuously the same, but that, while there were some rather marked individual differences, there was, on the whole, a surprising agreement among the substances of the respective groups in the directions, time, and degree of the changes in this property.

Particularly notable are (1) The great reduction in limestone requirement during the first two weeks, a decrease on the part of the manurial mixtures to a point far below that of the control soil, in which the requirement underwent no change at this time; (2) a great increase, both absolute and relative, in the control soil in its requirement during the following two weeks; (3) the comparatively slight changes during the second and third months, save in the case of the redtop mixture; (4) a second extensive increase in the requirement during the fourth and fifth months; (5) slight changes during the succeeding months,

except in the case of the redtop and sorrel mixture, with a majority of slight increases during the sixth and seventh months, and a slight but usually greater decrease during the next two months.

It is particularly notable that there was no difference in tendency at these periods among the legumes, nonlegumes, and stable manures.

The several averages of Table III show the net effects of the several additions upon the reaction of the acid soil used as a basis.

The control soil without manurial addition gradually but slightly increased in acidity after five months' exposure under the conditions of this experiment. The addition of either the acid green manures or of alkaline stable manure caused, within two weeks, a very great decrease in limestone requirement. This lowered requirement was very pronounced for a very short time, however; and in a month the requirement had returned nearly to the level of that for the control soil. A later phase, manifest after the third month, showed a greater acidity or lime requirement for the manurial mixtures than for the soil; and with a few exceptions this continued until the end of the experiment.

The average conditions of lime requirement throughout the experiment of the individual manurial substances, after the fermentations had well started, were as follows:

	Pounds per acre.		Pounds per acre.
Red clover	2,648	Hairy vetch	4,767
Sorrel	3,271	Canada field pea	4,987
Stable manure	4,275	Oats	5,308
Rye	4,317	Soybean	5,053
Alfalfa	4,326	Sweet clover	5,450
Redtop	4,349	Rape	5,500
Timothy	4,359	Corn	5,919
Control soil	4,741	Wheat	5,926

In general, red clover as a green manure was the best corrective of acidity, taking the whole period into account, and sorrel was the only green manure whose addition kept the soil, at all times after fermentation had started, below the level of that exhibited by the soil alone. On the other hand, wheat and corn increased the average lime requirement more than any other of the manurial substances tried.

CAUSES OF THE CHANGES IN LIMESTONE REQUIREMENT

When the causes to which the marked changes are due are considered, we may narrow the field of inquiry by these considerations:

(1) The periodicity of the changes is not a result of variation in the environment, in moisture relation, or in aeration, but is probably incident to successive series of fermentations with corresponding chemical products.

(2) The general similarity in the changes exhibited by all the manurial mixtures indicates that these changes are related to the constituents in which they are alike rather than to those in which they differ.

(3) The properties of the soil itself have no direct causal relation to these changes, since these changes are but slightly if at all manifest in the control soil. The changes are therefore related either to the substances possessed in common by the manures, or to other substances produced by reaction between the plant materials and the constituents of the soil.

CHANGES OF THE FIRST PERIOD

This 2-week period immediately succeeding the beginning of the experiment shows no change in the limestone requirement of the control soil, but a very marked reduction in that of all the manurial mixtures with the soil. This reduction has been so great as not only to destroy the immediate acid actions of the green manures but also a large part of those which the soil by itself exerts.

Coville, in his study of the reactions occurring during the formation of leaf mold, has outlined the causes of the changes manifest in that case. The same chain of effects, other than such as are due to leaching, are doubtless to be found in the case of the green manures.

The alkaline effects so promptly displayed may be accounted for by three well-known fermentative changes: (1) The destruction of the free acids of the green manures, probably by molds; (2) the destruction of a part or all of the combined organic acids, liberating the alkaline substances with which they were combined in the fresh plant material, so that these alkaline materials can aid in turn to satisfy part of the original requirement of the soil; (3) the conversion of the nitrogenous material of the manures to ammonia, which can, like the bases of the organic salts, serve as alkali for the soil.

No analytical studies suited to determine whether any or all of these causes had actually operated in these experiments were made. We have, however, some indirect evidence upon the subject.

In the first place, the surfaces of the mixtures were promptly covered with thick felts of white mold. The presence of organisms capable of rapid destruction of the organic acids of the green manures is therefore established.

In the second place, the degree of the change in limestone requirement and the neutralizing effect possible from the complete ammonification of all the nitrogenous substances of the manures, and that possible from the complete liberation of all the alkaline ash constituents of the manures, may exhibit relations serviceable for this study. These facts are set forth in Table V, the ammonia and alkaline ash figures being expressed in the calcium-carbonate equivalent for 20 tons of dry matter of the respective manures, the proportion mixed with the soil in these experiments.

TABLE V.—*Alkalinity and ammonia production in soils by green manures*

[Results expressed as the calcium-carbonate equivalent for 20 tons of dry manure]

Material.	Decrease in limestone requirement.	Ammonia equivalent.	Ash alkali equivalent.
	Pounds.	*Pounds.*	*Pounds.*
Control soil	0	.....	.....
Legume mixtures:			
Soybean	1,593	3,970	2,768
Canada field pea	2,543	5,212	3,120
Sweet clover	2,503	4,455	2,108
Alfalfa	2,473	3,941	3,128
Red clover	3,950	2,770	2,252
Hairy vetch	2,420	4,056	1,308
Subaverage	2,580	4,067	2,447
Nonlegume mixtures:			
Wheat	1,135	5,069	2,400
Rape	1,377	6,840	4,192
Oats	4,244	4,398	2,140
Corn	2,942	3,884	1,880
Rye	2,503	1,899	508
Timothy	2,033	1,842	928
Redtop	1,870	1,528	640
Sorrel	4,004	2,827	1,468
Subaverage	2,511	3,536	1,769
Barnyard-manure mixture	1,457	2,242	2,208
Grand average	2,468	3,662	2,070

In the average case, therefore, the nitrogen in the added manure would suffice to work the observed change if it were fully converted to ammonia within the brief period of two weeks. On the other hand, the ash constituents of the manure would, even though completely liberated, not suffice to work the observed change.

There are exceptions to these deductions from averages. In the cases of red clover, rye, timothy, redtop, and sorrel, the ammonia equivalent to the total nitrogen of the green manures would not suffice to effect the observed change. Indeed, in the case of the rye, the alkali equivalent of the rye, nitrogen, and ash together would not account for the entire change observed.

Moreover, there is little probability of so complete conversion of the manurial substances within two weeks as this comparison requires.

Some notion of the behavior of this acid soil with respect to the fermentative process of ammonification is afforded by the studies of Dr. G. C. Given, of the Pennsylvania Station. He found that the soil of plot 32 (the source of the soil used in the present experiment), first sterilized by heat and then inoculated by the bacteria from neighboring fer-

tile soil, changed to ammonia in seven days from one-third to one-half of the nitrogen added as dried blood or as cottonseed meal. In an earlier experiment the same investigator found that the bacteria from the soil of plot 32, when used to inoculate another previously sterilized soil, acted with like ammonifying vigor as those of the first mentioned of these two experiments.

Without attempting to apply these proportions to the conditions of our own experiments we may at least infer that during the first two weeks a very advanced stage of ammonification might be reached in the case of the highly fermentable fresh green manures.

INCREASE OF LIME REQUIREMENT AT LATER PERIODS

The facts presented in Tables III and IV show, as already has been pointed out, several general increases of limestone requirement in the manurial mixtures.

It is well known that among the oxidation processes of general occurrences in ordinary plowed land is that bacterial fermentation which results in the transformation of ammonia to nitric acid. The effect of such a change is clearly to increase the limestone requirement, because the nitric acid must combine with basic materials to form neutral salts. The degree of effect varies, however, for each unit quantity of nitric acid produced, according to the conditions in which the requisite nitrogen exists at the beginning of the period of observation. If, on the one hand, it is present in a body that has little or no effect on the reaction of the soil medium, then the conversion to nitric acid will change the acidity only in such measure as the nitric acid itself contributes to the acidity; on the other hand, if the nitrogen is already present as ammonia, it serves in the measure of its quantity to reduce the limestone requirement at that time, but upon conversion to nitric acid it not only adds to the acid but subtracts in like measure from the previous series of the alkaline substances, so that, in this case, it works a double effect. In the former of these alternatives 28 pounds of nitrogen would make enough nitric acid to require 100 pounds of carbonate of lime for its neutralization; in the latter case the same quantity of nitrogen in the form of ammonia would perform the neutralizing service of 100 pounds of carbonate of lime, but upon conversion to nitric acid would not only lose its former power but would require the direct action of an equal quantity of the calcium carbonate, so that the net effects of the conversion of the 28 pounds of ammoniacal nitrogen to nitric nitrogen are to increase the calcium carbonate duty of 200 pounds. There are several other alternative conditions possible. In one ammonia might be formed from a neutral body at the same rate as the nitric acid itself is generated. Of course, in this case the one substance exactly balances the other in its effect upon the limestone requirement.

Tables VI and VII give the quantities of nitric nitrogen present in our experimental materials at the second period of observation and the changes in these quantities during the later intervals. The determinations began with the 4-week period.

TABLE VI.—*Nitric nitrogen in manured soils (Series I)*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Material.	Period 2 (4 weeks).	Period 3 (3 months).	Period 4 (5 months).	Period 5 (7 months).	Period 6 (9 months).	Average through last 8 months.
Control soil	26.54	40.84	40.88	57.50	62.70	45.68
Legume mixtures:						
Soybean	46.00	70.84	136.24	136.98	143.06	106.62
Canada field pea	51.10	115.92	127.02	115.00	139.98	109.80
Sweet clover	21.52	96.02	115.00	169.46	157.04	111.80
Alfalfa	26.48	111.08	131.42	161.00	169.46	119.88
Red clover	3.58	33.94	184.00	184.00	246.00	130.62
Hairy vetch	39.52	58.88	105.10	128.80	140.32	94.52
Subaverage	31.37	81.11	133.13	149.21	149.31	112.21
Nonlegume mixtures:						
Wheat	76.36	138.00	147.20	238.50	207.46	161.50
Rape	60.70	128.72	216.48	214.64	207.48	165.80
Oats	21.52	81.74	118.68	134.18	153.18	101.86
Corn	43 04	98.12	115.12	153.32	161.00	114.12
Rye	14.36	44.16	58.88	80.50	86.98	56.96
Timothy	2.88	5.74	27.54	52.76	64.40	30.66
Redtop	7.64	11.96	42.92	53.64	68.48	36.92
Sorrel	1.76	15.04	48.44	59.62	80.50	41.06
Subaverage	28.53	65.56	96.91	123.64	133.68	88.61
Barnyard manure mixture	51.06	53.82	79.54	84.74	107.32	75.28
Poultry manure mixture [a]	([b])	176.64	245.32	214.64	257.60	223.40
Grand average	31.17	71.00	110.24	131.28	138.18	97.16

[a] Omitted from grand average. [b] Lost.

By reference to the grand average of Table IV, it is seen that, in general, the second 2-week period and that covering the fourth and fifth months were marked by great increases in the lime requirement of most of the manurial mixtures.

The data for nitric nitrogen exhibit no corresponding increase in the formation of nitrates, or at least in their accumulation, even if we assume that all found at the end of the fourth week were formed in the last 14 days of that time, which is not probable.

TABLE VII.—*Periodic changes in nitric nitrogen (Series I)*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Material.	Second and third months.	Fourth and fifth months.	Sixth and seventh months.	Eighth and ninth months.
Control soil	14.28	0.06	16.62	5.20
Legume mixtures:				
Soybean	24.84	65.40	.74	6.08
Canada field pea	64.82	11.10	−12.02	24.98
Sweet clover	74.50	18.98	54.46	−12.42
Alfalfa	84.60	20.34	29.58	8.46
Red clover	30.36	150.06	0.00	62.00
Hairy vetch	19.36	46.22	23.70	11.52
Subaverage	49.75	52.02	16.08	16.77
Nonlegume mixtures:				
Wheat	61.64	9.20	91.30	−31.04
Rape	69.02	86.76	−1.84	−7.16
Oats	60.22	36.74	15.50	19.00
Corn	55.08	17.00	38.20	7.68
Timothy	2.86	21.80	25.22	11.64
Rye	29.80	14.72	21.62	6.48
Redtop	4.32	30.96	10.72	14.84
Sorrel	13.28	33.40	11.18	20.88
Subaverage	37.02	31.32	26.49	5.29
Barnyard-manure mixture	2.76	25.72	5.50	22.58
Poultry-manure mixture [a]	(?)	68.68	−30.68	42.96
Grand average	39.83	39.23	20.90	11.03

[a] Omitted from grand average.

In the second place, even under the assumption according to which the nitrification would most largely increase the limestone requirement—namely, that the nitrates were formed from the previous stock of ammonia, and that the ammonification was entirely completed prior to the period in question—the quantities of nitric nitrogen found are altogether insufficient to account for the large development of the limestone requirement. Thus, if the 31.17 pounds of nitric nitrogen found at the end of the fourth week are assumed to have formed during the preceding 14 days, and it is further assumed that ammonification was arrested during that time, the corresponding increase of the average limestone requirement would be only 222.5 pounds, as compared with the observed increase of 1,261 pounds; and, in this way, the 39.23 pounds increase of nitric nitrogen during the fourth and fifth months could account for no more than 280 pounds of the 1,596 pounds increase in limestone requirement observed for that period.

The further fact that the second and third month witnessed a decrease in the limestone requirement and that this condition prevailed also, in the average case, during the eighth and ninth months, whereas nitric nitrogen

increases occurred during these two periods, is also illustrative of the lack of correspondence between the degrees of soil acidity, or limestone requirement, and of nitrification in these experiments.

While, therefore, nitric nitrogen is contributory to the effect in all soil acidity, it is by no means the chief factor producing that effect in these cases.

In passing, we may note several other points upon which these experiments throw some light. The average legume yielded in nine months more nitrates than the average nonlegume green manure, and both gave more than stable manure, but less than poultry manure. At the top of the list, exclusive of poultry manure, are red clover, wheat, and rape; at the bottom, soybeans, timothy, and redtop. Among the nonlegumes, wheat and rape held the most nitrogen and had potentially the most alkaline mineral matter, but red clover had, among the legumes, the least nitrogen and by no means the most alkaline ash. These properties were therefore not the factors that determined the respective rates and degrees of nitrification.

On comparing the manurial mixtures with the control soil, it appears that at the end of the first month, there was, on the average, little difference between the manured and unmanured soils; but the departures from the average are relatively numerous. The red-clover mixture had less nitrates than the control soil until after the third month, but greatly exceeded it thereafter; and the timothy, redtop, and sorrel mixture did not catch up to the untreated soil for about five months, and thereafter differed little from it; in all other cases than the last three named, however, the manured soils surpassed the control soil in nitrate accumulation.

These cases are simply cumulative with the other laboratory and field studies previously made upon these quite highly acid or lime-requiring soils from plot 32, as to the fact that their acid properties do not prevent a fairly vigorous nitrification. With respect to this point, the accumulation of nitrates in the wheat mixture during the last four months of the experiment, when its limestone requirement was upward of 7,000 pounds to the acre 7 inches is to be especially noted.

SERIES II: AIR-DRIED GREEN MANURES

For Series II the same leguminous plant material except Canada field peas was used as in Series I, but mixed air-dry instead of fresh with the soil; of the nonlegumes only five materials were used in the air-dry state—namely, rape, corn, rye, redtop, and sorrel. Aside from the air drying of the green manures, the experiments of Series II were like those of Series I, except that the limestone requirements and nitric nitrogen were not determined at the 4-week and 5-month periods.

The results of the several determinations made are given in Tables VIII to XI.

TABLE VIII.—*Limestone requirement of air-dried green manures (Series II)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	Period 1 (2 weeks).	Period 3 (3 months.)	Period 5 (7 months).	Period 6 (9 months).	Average for 9 months.
Control soil	4,644	4,644	4,871	4,904	4,765
Legume mixtures:					
Soybean	3,860	4,169	5,568	5,722	4,817
Sweet clover	3,325	5,515	5,345	6,536	5,179
Alfalfa	3,965	4,939	5,481	5,790	5,043
Red clover	1,763	2,237	4,203	3,626	2,957
Hairy vetch	4,847	5,277	6,366	6,265	5,688
Subaverage	4,400	4,427	5,393	5,588	4,737
Nonlegume mixtures:					
Rape	4,871	5,790	5,616	5,515	5,448
Corn	3,321	5,210	6,910	6,944	5,596
Rye	3,592	3,897	5,616	5,960	4,766
Redtop	3,288	4,304	5,176	5,855	4,656
Sorrel	3,187	3,085	4,813	5,481	4,141
Subaverage	3,652	4,457	5,626	5,951	4,921
Grand average	4,066	4,442	5,509	5,769	4,829

TABLE IX.—*Changes in the limestone requirement of soils (Series II)*

[Results expressed as pounds of limestone per 2,000,000 pounds of soil]

Material.	First two weeks.	From end of second week to end of third month.	From end of third to end of seventh month.	Eighth and ninth months.
Control soil	0	0	227	33
Legume mixtures:				
Soybean	−403	−957	309	154
Sweet clover	−511	−1,858	2,194	1,191
Alfalfa	−345	−1,078	974	309
Red clover	−1,706	−1,194	474	−577
Hairy vetch	386	−841	430	−101
Subaverage	−516	−1,186	876	195
Nonlegume mixtures:				
Rape	−234	−577	919	−101
Corn	−1,278	−2,275	1,889	−34
Rye	−991	−1,174	305	344
Redtop	−62	−1,368	1,016	679
Sorrel	−1,592	−954	−102	668
Subaverage	−831	−1,270	805	311
Grand average	−674	−1,228	841	253

TABLE X.—*Nitric nitrogen in air-dried green-manured soils*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Material.	Period 3 (3 months).	Period 5 (7 months).	Period 6 (9 months).	Average for 9 months.
Control soil	40.84	57.50	62.70	53.68
Legume mixtures:				
Soybean	98.12	128.80	161.00	129.30
Sweet clover	53.82	107.18	123.82	94.94
Alfalfa	98.90	129.08	169.46	132.48
Red clover	52.54	86.98	107.32	82.28
Hairy vetch	58.88	107.18	128.80	98.28
Subaverage	72.25	111.84	138.08	107.46
Nonlegume mixtures:				
Rape	118.46	173.88	238.50	176.94
Corn	55.20	107.18	119.14	93.84
Rye	35.60	64.40	68.50	56.16
Redtop	24.52	46.00	58.41	42.78
Sorrel	26.96	78.52	80.50	61.98
Subaverage	52.15	95.00	113.01	86.34
Grand average	62.20	102.92	117.44	96.90

TABLE XI.—*Changes in nitric nitrogen of air-dried green manures*

[Results expressed as pounds of nitric nitrogen per 2,000,000 pounds of soil]

Materials.	End of third to end of seventh month.	During eighth and ninth months.
Control soil	16.66	5.20
Legume mixtures:		
Soy bean	30.68	32.20
Sweet clover	53.36	16.64
Alfalfa	30.18	40.38
Red clover	34.44	23.34
Hairy vetch	48.30	21.62
Subaverage	39.39	26.64
Nonlegume mixtures:		
Rape	55.42	64.62
Corn	51.98	11.96
Rye	28.80	4.10
Redtop	21.48	12.41
Sorrel	51.56	1.98
Subaverage	41.85	19.01
Grand average	40.62	23.32

Since the same materials throughout were not used in Series I and II, the figures from the analyses of the mixtures of those materials used in

Series I, that were also used in Series II, and representing likewise the same periods as are covered by the figures of Series II, have been grouped in the following tabulations (Tables XII and XIII):

TABLE XII.—*Average limestone requirement and nitric nitrogen in manured mixtures*

[Results expressed in pounds per 2,000,000 pounds of soil]

Materials.	Limestone requirement.						Nitric nitrogen.			
	Original.	Period 1 (2 weeks).	Period 3 (3 months).	Period 5 (7 months).	Period 6 (9 months).	Average for months.[a]	Period 3 (3 months).	Period 5 (7 months).	Period 6 (9 months).	Average for months.[a]
Control soil	4,644	4,644	4,644	4,871	4,904	4,766	40.84	57.50	62.70	53.68
Fresh green manure:										
Legume	5,253	2,664	3,532	5,775	5,136	4,277	54.15	156.05	151.18	120.69
Nonlegume	5,789	3,214	3,980	5,793	5,251	4,560	59.80	112.34	120.89	97.68
Air-dry green manures:										
Legume	5,253	4,480	4,427	5,393	5,588	4,737	72.25	111.84	138.08	107.39
Nonlegume	5,789	3,652	4,457	5,626	5,951	4,921	52.15	94.00	113.01	86.39
Stable manure	(?)	3,187	3,797	5,549	4,575	4,277	53.82	84.74	107.32	81.96

[a] The averages in the last column of this table represent only the data for the third, fifth, and sixth periods.

TABLE XIII.—*Average changes in limestone requirement and nitric nitrogen*

[Results expressed in pounds per 2,000,000 pounds of soil]

Materials.	Limestone requirement.				Nitric nitrogen.	
	First 2 weeks.	End of second week to end of third month.	End of third to end of seventh month.	Eighth and ninth months.	End of third to end of seventh month.	Eighth and ninth months.
Control soil	0	0	237	33	16.66	5.20
Fresh green manures:						
Legume	−2,589	868	2,243	−941	101.90	−4.87
Nonlegume	−2,575	766	1,813	−691	52.54	8.55
Air-dry green manures:						
Legume	−773	−53	1,966	−851	39.59	26.24
Nonlegume	−2,137	805	1,169	−1,031	41.85	19.01
Stable manure	(?)	610	1,752	−974	30.92	22.58

It is evident from Table XII that the fresh green manures affected the limestone requirement in greater degree and for a longer period than the corresponding air-dried green manures; that the changes in lime requirement after the first period of fermentation were not so great in case of the air-dried as in that of the fresh material; and that, with a single exception, this requirement did not reach so high a point with the air-dried materials as with the fresh, the exception noted being for the nonlegume materials in the last months of the experiment. The limestone requirement of the air-dried mixtures, as contrasted with that of the soil untreated, was in general about on a level with the latter until the end of the third month, after which it became considerably higher.

As to nitrates, the accumulation did not reach so high a point during the time of the experiment with the air-dried manures as with the fresh.

In general, the evidence indicates that none of the major fermentations here concerned progressed as rapidly with the air-dried as with the fresh materials. Wollny[1] has observed a like decrease in the rapidity of fermentation with fresh plants air-dried and remoistened.

ORGANIC MATTER OF MANURED SOILS

The quality of sourness, or acidity, is sometimes traceable to the condition of the mineral constituents of soils sometimes to that of their organic constituents. In the case of the soil used for these experiments, the factors conditioning its acid or lime-requiring qualities have not been at all completely worked out.

It is, however, a silty soil rather than one rich in clayey constituents, and on the fertilizer plots, other than those treated with ammonium sulphate, has not exhibited a high-degree lime requirement, though, elsewhere in the vicinity, when under grass for a long time or even long farmed by the common rotation, it gradually acquires acid properties.

In the case of the present experiment, however, the differences exhibited as the result of the addition of manures chiefly composed of organic substances must be due, either directly to the fermentation residues of these manures or indirectly to changes they induce in the soil constituents, organic or inorganic.

To gain some idea of some of the relationships of the organic residues from the green manures, the several mixtures were each thoroughly remixed, passed through a sieve of 40 meshes to the inch, and submitted to additional analytical examination.

ORGANIC MATTER

The amount of the organic matter in the soils was approximated by ascertaining the loss in weight which they exhibited upon burning, correction being made for the portion of this loss that is due to the hygroscopic moisture of the respective soils.

It is recognized that the method of determination here used gives too high direct results, owing to the fact that the water of hydration of the mineral matters, the carbon dioxid of the carbonate, part of the sulphur of the sulphids, and part of any chlorin present probably escape with the gases formed from the burning of organic matter. We are here chiefly interested, however, in differences in mixtures resulting from the use of a single soil as the chief constituent, of which mixture it constituted slightly over 98 per cent of the dry matter. These differences are not, it is believed, considerably affected by the sources of error just named.

[1] WOLLNY, Ewald. DIE ZERSETZUNGEN DER ORGANISCHEN STOFFE UND DIE HUMUSBILDUNGEN. p. 115. Heidelberg, 1917.

In Table XIV are stated (1) the corrected loss of the several soils in burning, (2) the excess of such loss shown by the manurial mixture as compared with the unmanured soil, (3) the corresponding weights of these excesses in 2,000,000 pounds of the soil, (4) the weights of organic matter originally added in the manurial substances, (5) the weights, and (6) the percentages of the added organic matter that had disappeared during the nine months of the experiment.

TABLE XIV.—*Total organic matter of the manured soils (Series I)*

Material.	Losses on burning.	Excess of losses by manured *v.* control soil.	Weight of excess in 2,000,000 pounds.	Weight of manurial organic matter originally added.	Loss of added organic matter by fermentation.	
					Weight.	Per cent.
	Per cent.	*Per cent.*	*Pounds.*	*Pounds.*	*Pounds.*	
Control soil	4.873	.....	.....	.....	.....	.....
Legume mixtures:						
Soybean	5.304	0.426	8,520	29,216	20,696	70.84
Canada field pea	5.479	.669	13,380	35,540	22,160	62.35
Sweet clover	5.482	.604	12,080	36,864	24,784	67.23
Alfalfa	5.346	.468	9,360	36,548	27,188	74.39
Red clover	6.112	1.234	24,680	37,100	12,420	33.48
Hairy vetch	5.535	.657	13,140	37,216	24,076	64.69
Subaverage	5.543	.676	13,527	35,414	21,887	61.80
Nonlegume mixtures:						
Wheat	5.547	.669	13,380	35,744	22,364	62.57
Rape	5.315	.437	8,740	32,132	23,392	72.80
Oats	5.539	.661	13,220	35,384	22,164	62.64
Corn	5.419	.541	10,820	36,516	25,696	70.37
Rye	5.812	.939	18,780	38,160	19,380	50.81
Timothy	5.746	.868	17,360	37,504	20,144	53.71
Redtop	5,644	.766	15,320	37,596	22,276	59.25
Sorrel	5.455	.577	11,540	36,072	24,532	68.01
Subaverage	5.560	.682	13,645	36,138	22,483	62.21
Barnyard manure mixture	5.563	.685	13,700	24,352	10,652	43.74
Poultry manure mixture [a]	5.368	.490	9,800	28,552	18,752	65.68
Grand average	5.553	.680	13,601	35,063	21,462	61.21

[a] Omitted from grand average.

With respect to the soils of Series I, these general facts appear: All the organic manures left the soil richer in organic matter nine months after their addition. In the application of this result to practice it must be recalled that the moisture condition was most favorable and that the temperatures were also somewhat more favorable to fermentation and decomposition than a corresponding nine months outdoor season from March to November would be. Furthermore, it is to be recalled that the soil was a fine silty loam, and was, on the one hand, less favorable

to decomposition than a loose sandy soil would be, and more favorable, on the other, than would be the case with a compact clay soil.

TABLE XV.—*Total organic matter of the green-manured soils (Series II)*

Material.	Losses on burning.	Excess of losses by manures *v.* control soil.	Weight of excess in 2,000,000 pounds.	Loss of added organic matter by fermentation.	
				Weight.	Per cent.
	Per cent.	*Per cent.*	*Pounds.*	*Pounds.*	
Control soil	4.878	.....	.....	.....	.....
Legume mixtures:					
Soybean	5.465	0.587	11,740	17,476	59.82
Sweet clover	5.541	.663	13,360	23,504	63.33
Alfalfa	5.586	.708	14,160	22,388	61.25
Red clover	5.740	.826	16,520	20,580	55.47
Hairy vetch	5.720	.843	16,860	20,356	54.70
Subaverage	5.611	.733	14,660	20,861	58.73
Nonlegume mixtures:					
Rape	5.408	.530	10,600	21,532	67.01
Corn [a]	.....	.....	.....	.....	.....
Rye	5.620	.742	14,840	23,320	61.11
Redtop	5.723	.854	17,080	20,516	54.57
Sorrel	5.698	.821	16,420	19,652	54.48
Subaverage	5.612	.734	14,680	21,255	59.15
Grand average	5.612	.734	14,670	21,036	58.92

[a] Sample lost.

There was no appreciable difference in the proportion of loss of the organic matter of the groups of legumes and nonlegumes, respectively, and both suffered greater loss than stable manure, which had doubtless been considerably changed by fermentation before application.

There was, however, a good deal of difference in the degree of loss exhibited by individual materials. The figure for red clover is surprisingly low, and those for rye and timothy are much below the average.

We may question whether there is any direct relation between the amount of total residual humus and the limestone requirement at the end of the nine months period. We are at once confronted by the fact that where alfalfa, red clover, hairy vetch, rye, timothy, redtop, and sorrel were added to the soil, its limestone requirement was less than that of the untreated soil, and yet in each of these cases there was a large residue in the soil of organic matter from the green manures. We may, therefore, not regard the organic residues as active, as a whole, to cause the observed acidity. When we consider the variety of organic matters originally present, and the differences in the mutual proportions they originally bore to one another, it would at once appear highly improbable that the residues from their fermentation would have like acid effects for equal units by weights.

Considering the fresh and air-dried green-manure mixtures as two groups, we find, if we include in these groups only those materials represented in both series, these differences:

	Applied fresh.	Applied air-dry.
Excess of organic matter over soil:		
Per cent	0.679	0.734
Pounds in 2,000,000	73,580	74,680
Loss by soil fermentation:		
Pounds in 2,000,000	22,083	20,976
Per cent	61.91	58.63

That is, at the end of nine months there was lost by fermentation about 1,100 pounds more of the organic matter applied in a fresh green state than in an air-dried green state. This amounts to but 3.3 per cent of the total application of organic matter. The delayed fermentation of the air-dried material, the rather marked effect of which fermentation difference with respect to both limestone requirement and nitrate development has received earlier comment, resulted finally in much less difference in total decomposition than might have been forecast from the soil acidity and nitrification differences.

Several years ago in studying the grass roadways bounding the several tiers of the General Fertilizer Series of plots, it was found that these roadways differed much in their lime requirement, and that in each instance the lime requirement bore a fixed ratio to the amount of the free humic acid—that is, that portion of the humus that was soluble directly in weak alkali without the previous removal of lime, etc., by acid washing. The constant ratio observed in that grass-land soil was 11.27 of the free humic acid to 1 of lime.

To determine whether these green-manuring soils would exhibit a like condition with the grass lands mentioned above and also more generally to ascertain whether the humus of the several green-manuring soils, as they existed nine months after the application of the manures, was possessed of peculiarity in the proportion of alkali-soluble humus—sometimes called "active humus"—this group of constituents was determined by a modification of the McBride method in which the filtration of the alkaline liquid was performed by the aid of Chamberland-Pasteur filters, which effectually remove suspended matters and yield a clear filtrate. The weakly alkaline solution was made to act upon the soil in two conditions:

A.—Ten gm. freed from basic materials by washing with 1 per cent of hydrochloric acid and then with water was shaken for 15 hours in a Wagner shaking apparatus with 1,000 c. c. of 4 per cent ammonia water, the suspended matter allowed to settle, the overlying liquor then filtered in the manner just stated, and an aliquot of the filtrate evaporated in a weighed platinum dish and dried at 103° C. for four hours. The material

was then burned in this dish, the loss in weight counted as free and combined humic acid, and the mineral residue as humus ash.

B.—An equal weight of the soil was treated in exactly the same way, except that the removal of the bases by washing with hydrochloric acid and water was omitted. The loss on burning the alkali-soluble material obtained in this manner was counted as free humic acid and the associated humus ash determined as in the preceding case.

It is to be observed that if any portion of the organic matter is soluble in either weak hydrochloric acid or water, or both, and also in weak ammonia water, a loss of this fraction of the material would occur with the treatment by the usual method, A, but not by method B. In such case these two treatments would not give the full measure of the material, free and combined with basic matter, that is capable of solution in the weak alkali.

TABLE XVI.—*Determinations of humus and humus ash in experiment mixtures (Series I and II)*

Material.	Fresh manures.				Air-dry manures.			
	Humus ash.		Soluble humus.		Humus ash.		Soluble humus.	
	A	B	A	B	A	B	A	B
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Control soil	0.48	0.57	1.23	1.48	.....	.....	.....	.....
Legume mixtures:								
Soybean	.....	.....	1.30	1.80	0.45	0.40	1.39	1.64
Canada field pea	.....	.....	1.35	1.65	.....	.....	.....	.....
Sweet clover	.....	.....	1.32	1.66	.41	.44	1.41	1.65
Alfalfa	.....	.....	1.59	1.70	.40	.47	1.40	1.69
Red clover	.....	.....	1.61	1.88	.43	.94	1.38	1.67
Hairy vetch	.....	.....	1.40	1.63	.35	.45	1.44	1.74
Subaverage	.....	.....	1.43	1.72	.41	.54	1.40	1.68
Nonlegume mixtures:								
Wheat	.....	.....	1.36	1.81	.....	.....	.....	.....
Rape	.....	.....	1.25	1.61	.38	.53	1.33	1.59
Oats	.....	.....	1.39	1.75	.....	.....	.....	.....
Corn	.....	.....	1.35	1.63	.....	.....	.....	.....
Rye	.....	.....	1.41	1.78	.49	.47	1.41	1.65
Timothy	.....	.....	1.48	1.66	.....	.....	.....	.....
Redtop	.....	.....	1.38	1.61	.33	.51	1.50	1.73
Sorrel	.....	.....	1.38	1.65	.36	.53	1.43	1.66
Subaverage	.....	.....	1.37	1.69	.39	.51	1.44	1.61
Barnyard-manure mixture	.....	.....	1.33	1.70	.....	.....	.....	.....
Poultry-manure mixture [a]	.....	.....	1.27	1.55	.....	.....	.....	.....
Grand average	.....	.....	1.39	1.70	.40	.53	1.42	1.67
Fresh humus, subaverage:								
Legume	.....	.....	.....	.....	.....	.....	1.44	1.73
Nonlegume	.....	.....	.....	.....	.....	.....	1.35	1.66

[a] Omitted from grand average.

The comparison of the results obtained by the two methods of determining the alkali-soluble humus in this acid soil, and in its fermented mixtures with various organic manurial substances, shows strikingly that more organic material was obtained by the direct solvent action of ammonia (method B) than by washing with weak hydrochloric acid and water for the purpose of breaking up the humate combinations with basic substances and then treating with the dilute ammonia water (method A). The mineral matters associated with the dissolved humus are more abundant with the former treatment than with the latter; but even in the two individual cases where the reverse is true, the direct action of the ammonia removed more organic material.

From these facts we may conclude that in this acid soil, both untreated and as modified by fermentations of organic manures applied, very little of the "active humus" is combined with bases; that washing with weak acid and water removes a considerable amount of the organic matter and usually some basic materials.

It is further clear that the data obtained by these analytical methods are not such as to make it possible to establish a relation between the limestone requirements of the respective soils and a particular fraction of their active humus.

The facts set forth in Table XVI do not show any noteworthy difference between the soil percentages of active humus in the mixtures obtained from fresh as compared with the air-dried manures.

We may present, however, a comparison from the data of Tables XV and XVI to show the respective proportions in which the total humus in the several groups of manurial mixtures, in excess of that accounted for by the untreated soil, is composed of active humus as determined by the usual method A. The proportion shown by the soil itself is added for further comparison (Table XVII).

TABLE XVII.—*Percentage of active humus in the organic matter of the manurial mixtures*

Material.	Total organic matter (pounds in 2,000,000).	Active humus (pounds in 2,000,000).	Percentage of active humus in organic matter.
Untreated soil	97,560	24,600	25.19
Excesses contributed by—			
Legume green manures:			
Fresh	13,556	4,000	29.36
Air-dry	14,660	3,400	20.46
Nonlegume green manures:			
Fresh	13,595	4,800	35.31
Air-dry	14,680	4,200	28.61
Stable manure	13,700	2,000	14.60
Poultry manure	9,800	800	8.16

In case of both legume and nonlegume green manures, therefore, the organic residue from the material applied fresh contains a larger proportion of alkali-soluble humus than does that from the air-dried manure. The percentage of alkali-soluble humus in the organic residue from the fermentation of stable manure in the soil is much less and that from the poultry manure is even lower.

Finally, we may consider whether these examinations exhibit any definite relation between the limestone requirement due to the added green manure and the alkali-soluble organic residues from these additions. For such purpose the results from the determination of alkali-soluble humus by method B are possibly best suited.

It will not be needful to make an extensive comparison in order to discover that no definite relation of the kind stated is here apparent. Table II shows 8 cases out of 15 in which the limestone requirements of the manured soils was less than that of the untreated soil at the end of the 9-month period; Table VIII shows one such case out of 10. On the other hand, Table XVI shows no case in which the alkali-soluble humus determined by method B in the manured soils is not greater than that of the untreated soil.

Therefore, if the chief acid effect in these cases is due to the alkali-soluble humus constituents as a whole, the influence of a unit weight of these parts of the several organic residues must be very different in degree in the respective cases.

SUMMARY

Under conditions corresponding for nine months to those of a summer fallow with fairly frequent, thorough cultivation and well-distributed rainfall of amount just sufficient to keep the soil well moist without setting the drains aflow, the following facts were observed with respect to the acid soil of the ammonium-sulphate plot 32, both as fallowed by itself, with stable manure and with various leguminous and nonleguminous manuring crops, applied fresh and also in an air-dry condition, respectively.

I.—THE SOIL WITHOUT MANURIAL ADDITION

(1) Its limestone requirements changed little, if at all.

(2) Its nitrate content increased sharply at two periods, first, during the second and third months, and at a less rate after the fifth month. If these increases represented a change from ammonia to nitric acid, without a simultaneous replacement of the transformed ammonia from organic nitrogenous sources, they would be equivalent to increases in the limestone requirement of 104 and 120 pounds, respectively, to the acre 7 inches (2,000,000 pounds).

(3) At the conclusion of the experiment the amount of nitric nitrogen was 36.16 pounds greater than at the beginning, or somewhat more than double.

II.—THE SOIL WITH STABLE MANURE ADDED

(4) The original limestone requirement of this mixture was not determined, nor were the data needful for its computation secured. There was, however, an almost continuous increase in this requirement from the end of the second week to the conclusion of the seventh month. During the third and fourth weeks the increase was 678 pounds (in 2,000,000), and during the fourth to the seventh month, inclusive, 1,652 pounds; but the two following months witnessed a decrease of nearly 1,000 pounds.

(5) The nitrates, on the other hand, increased continuously after the fourth week. This increase was greatest during the fourth and fifth months and again during the eighth and ninth months; at the one time the limestone requirement was increasing, and in the other markedly decreasing. At the utmost of its effect the nitrification can account for but a small fraction of these limestone-requirement changes.

(6) Until the end of the third month the limestone requirement of the stable manure mixture was less than that of the soil; from the fifth to the seventh month it was 1,000 pounds greater than that of the soil alone; but at the end of the experiment the soil had the greater lime requirement.

(7) At all times the stable manure mixture showed more nitrates than the soil alone.

III.—THE SOIL WITH ADDED FRESH GREEN MANURES

(8) At the end of the first two weeks the limestone requirement of these mixtures was, on the avearge, about 1,400 pounds less (in 2,000,000) than that of the soil alone. During these two weeks this requirement decreased from 5,858 pounds, the theoretical original acidity of the mixtures, to 3,044 pounds, a decrease of 2,540 pounds, or nearly one-half. As noted earlier in this summary, the soil alone showed no change in this requirement during this period.

(9) The plan of the experiment was not sufficiently complete to afford a proof of the cause of this decreased limestone requirement. Three factors may have operated to cause it: A destruction of the free acids of the green manures; a destruction also of the plant acids combined as salts with a consequent liberation of alkaline ash constituents; and the conversion of the organic nitrogen to ammonia. The first factor is too small to account for a large fraction of the observed decrease. Potentially, either of the others would suffice to account for it in most of the cases, though not in all. Complete destruction of the acid combinations in the plants within two weeks, or complete ammonification of the organic nitrogen within two weeks, seems unlikely. It is probable, therefore, that a large development of the three factors at the same time is the real explanation of the observed change.

An increase of limestone requirement in the case of these mixtures occurred during the third and fourth weeks, and again during the fifth

to sixth months, followed in the average case by but a small decrease during the eighth and ninth months. In 7 out of the 14 mixtures with fresh green manures a slight depression of the limestone requirement appeared at the end of the third month, and in but one of the remaining cases was there an important increase during this time.

(10) In the average case, though with a number of exceptions, the soil alone had a greater limestone requirement than its fresh green manure mixture up to the end of the third month, but from the end of the fifth month until the end of the experiment these mixtures on the average had the higher requirement.

(11) The course of the limestone requirement varies, however, quite differently in the several individual cases. With red clover it dropped to nothing by the end of the third month, and then jumped within the next two months to nearly 5,000 pounds, the greatest and most abrupt change appearing in these records. The highest individual limestone requirement was that of wheat at the end of the fifth month—namely, 7,594 pounds. The sorrel mixture was the only one that did not at some time exceed the soil in its limestone requirement.

(12) Among the individual green manures the highest limestone requirements on the average throughout the experiment were those of corn among the nonlegumes and of sweet clover among the legumes; the lowest, those of sorrel and red clover in these respective subgroups.

(13) In the average case these mixtures held at the end of the second week about as much nitric nitrogen as the soil; but thereafter the increase was continuous to the end of the experiment, when these mixtures held more than twice as much of this constitutent as the soil alone. In individual mixtures there were fluctuations in the quantity rather than a steady increase.

(14) The averages throughout the season show that the rape, wheat, and red clover mixtures had the most nitrates, while the timothy, redtop, and sorrel mixtures had less than the soil alone.

(15) In all these cases, as in that of the stable manure mixture, nitrification alone was absolutely insufficient to account for the observed developments of the limestone requirement.

(16) Although the green manured soil had an average limestone requirement much greater than that of the untreated soil, there was a quite strong nitrification in the presence of the green manures. In other words, here, as in the field, the acidity developed has not prevented fairly vigorous nitrate formation.

IV.—THE SOIL WITH ADDED AIR-DRY GREEN MANURES

(17) All the fermentative changes followed the same direction displayed by the mixtures with fresh green manures. The depression of the limestone requirement in the early weeks was less pronounced, the gain steadier but more delayed thereafter. The nitrification also progressed more slowly, and apparently had not reached its highest accumulation of

products at the time when the experiment ended. Under these conditions the red clover did not develop at any time a neutral soil; and at the end of the experiment all the mixtures, except that with red clover, had a greater limestone requirement than the soils alone exhibited.

V.—EFFECTS OF THE ORGANIC MANURES UPON THE AMOUNT AND CONDITION OF THE HUMUS SUPPLY OF THE SOIL

(18) The organic matter of the soil is the net result of manured and crop-root additions and the fermentative decomposition subtraction. With open soil, good moisture supply, summer temperature, and vigorous bacterial life, the additions may be matched by a greater subtraction, with no permanent gain in humus as the result. In the case of this silty loam, with such lime deficiency as is usually accompanied by a lowered activity of the fermentative soil organisms, a less rapid destruction of the organic matter should be expected.

(19) In every case the soils mixed with the various organic manures showed, nine months afterward, larger quantities of organic matter than the untreated soil.

(20) The amounts of these excesses vary much with the individual case, but on the average the amounts for legumes and nonlegumes, green manures and stable manures are well within the limits of analytical error. Soybeans, rape, alfalfa, and poultry manures left the smallest residues; red clover, rye, timothy, and redtop the greatest. The figures for the red clover residue, like those for its limestone requirement, are so peculiar that, although the results have been confirmed by repeated analyses, repetition of the experiment seems needful to establish their full correctness.

(21) The absence of a determination of total organic matter in the soil at the beginning of the experiment makes impossible the calculation of the total quantity lost by the soil and its mixtures during the experiment. By comparing the amounts in the mixtures in excess of that in the unmanured soil, at the end of the experiment, with the organic matter added in the manures, the amounts and proportions of the destruction of the added organic supplies can be approximated. This comparison indicates that, in the cases of the poultry manure and of the average green manures applied in a fresh condition, three-fifths of the added organic matter was destroyed by the soil ferments during nine months, while of the organic substance of the stable manure, partly rotted when applied, little more than two-fifths was destroyed. The largest proportion of destruction was 72.8 per cent in the case of rape; the lowest—omitting the peculiar red clover results from consideration—was 50.8 per cent in the case of rye

(22) These residual organic decomposition products, each considered as a whole, have no specific effect upon lime requirement, for while all the mixtures held at the close of the experiment more organic matter

than the unmixed soil, some of these mixtures had a greater limestone requirement than the soil itself, some a less.

(23) The green manures applied air-dry were not so largely decomposed as those applied in a fresh state. The former left about 1,000 pounds an acre more of organic residues than the former.

(24) Of the total organic residue in the untreated soil, only 25 per cent were left as "active humus" or humus soluble in dilute alkali, as determined by the usual method.

(25) Of the organic residues from the fresh green manures a somewhat larger proportion, 29 and 35 per cent for the legume and nonlegume groups, respectively, were left in "active humus" condition; but of the stable and poultry manures, much less, 14.6 and 8.2 per cent.

(26) In the mixtures with air-dry green manures the proportion of "active humus" was from one-third to one-fifth less than in the corresponding mixtures with fresh green manures.

(27) Of the "active humus" in these acid soil mixtures, little, if any, exists in ammonia-insoluble combinations with lime or other bases, and probably a considerable fraction can be dissolved by weak acid and water.

(28) The properties of these variously manured soils show no more definite relationship between their limestone requirement and the "free humic acid"—that is, the humus directly soluble in weak ammonia—than exists between the total organic matter and this requirement. If the material causes of the observed requirement belong to this major fraction of the active humus, either they are of very different composition and degree of influence or else they exist in very different quantities in the several free humic acid residues, a condition very different from that observed in old grass lands upon the same body of soil.

In general, these experiments have satisfactorily shown that fresh green manures plowed under on this acid silty loam soil reduce its acidity very soon after plowing under, but finally leave a soil of increased acidity; also that nitrification goes on in them quite vigorously under suitable moisture temperature and aerative conditions and that the green manured soils are rich in nitrates, despite the soil acidity. As to the cause of the increased acidity, beyond showing that it is not largely due to nitrification and indicating that it is in some way associated with the added organic materials or their fermentative residues, the experiments furnished little definite information.[1]

[1] The writer is indebted to Dr. Wm. Freer for his valuable assistance in the interpretation of results herein discussed.

A LEAFBLIGHT OF KALMIA LATIFOLIA

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INTRODUCTION

During the summer of 1914 a bed of mountain laurel (*Kalmia latifolia*) on the Department grounds became affected by a leafspot or blight. The progress of the disease in this bed was slow, but at the end of about 18 months there was not a single plant in the bed which did not show the typical brown margins and areas described below. The disease has also been found by the writer in Rock Creek Park and in the hills about Anacostia, D. C. Cross-sections of the affected leaves showed the presence of a very delicate fungus mycelium within the tissues. Cultural studies were begun and a fungus was isolated, which has been proved to be the cause of the disease.

DESCRIPTION OF THE DISEASE

The disease is characterized by a blight or dryrot involving large areas of the leaf blade or the entire leaf. Later, it extends through the petioles into the stems and may eventually kill the entire plant. Those leaves but slightly diseased show small, irregular, dark-brown spots scattered about over the blade. In fact, the disease in its early stages, especially on young, tender leaves, is visible only with a hand lens, appearing as tiny brown specks scattered about over an area of leaf surface which appears slightly lighter green than that of the surrounding healthy portion. These spots increase in size slowly, and finally become visible as tiny brown points in clusters, surrounded by a much lighter portion of green leaf tissue (Pl. 14, B). During dry, cold weather these diseased areas may remain as definitely outlined small spots for three to four weeks or longer; but if the air is moist and warm, they soon coalesce, forming the large, seal-brown to Vandyke brown [1] areas (Pl. 14, A, C), which finally involve the entire leaf blade. The incipient stage of the disease is most frequently seen at or near the tip, or along the margin of the leaf. Sometimes it spreads from the point of infection at the tip in rather an even line across the leaf blade, but it may also follow the midrib or extend along the margins of the leaf. In the latter case the margins usually curl, the inner portion of the leaf blade then appearing convex. The badly affected leaves, petioles, and stems become very dry and brittle, and such leaves drop off rather easily.

[1] The color terms mentioned throughout the text are according to Ridgway's Color Standards (Ridgway, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912).

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ISOLATION OF THE CAUSAL ORGANISM

Numerous free-hand sections of diseased leaves were made from what appeared to be the early stages of the disease—that is, the small brown spots and the adjacent portion of leaf tissue. In a few of these sections a delicate, frequently-branching mycelium was observed. Failure to find the fungus in a larger portion of the sections studied was undoubtedly due to the fact that it is not found far beyond the browned areas, the reduction of the chlorophyll beyond these areas being due possibly to toxic products of the fungus growth.

About a dozen of the diseased leaves in all stages of infection were placed without surface sterilization on moist filter paper in petri dishes, which were kept under large bell jars. Another set of dishes was arranged in the same way, except that the leaves were surface-sterilized by immersing them for one to five minutes in 1 to 1,000 mercuric-chlorid solution. The petri dishes and filter papers used were also sterilized. After a few days the leaves which had not been sterilized were covered with dense mycelial growths of a variety of fungi: Species of Alternaria, Pestalozzia, Cephalothecium, Aspergillus, etc. Numerous pycnidia were developing on a few of the leaves. Some of these were found to be the fruiting bodies of *Phyllosticta* spp. However, the majority did not appear to belong to this genus, but they were at this time too immature for examination. After about two weeks it was evident that they were not the pycnidia of *Phyllosticta* spp. Yellow spore masses in the form of cirri were extruding from them. These cirri were found to consist of spores of two kinds: An oval, hyalin, 1-celled spore with two or more definite oil drops, and a much longer, filiform, curved or hooked spore, also hyalin and 1-celled.

In the meantime those leaves which had been sterilized before being placed in the sterile moist petri dishes showed none of the abundant fungus mycelium present on the unsterilized leaves, but did show numerous pycnidia of the same type as some of those found on the latter, and they were also extruding the same kind of long, yellow cirri. These cirri were found to contain the two kinds of spores just described.

Petri-dish poured plates were made in the usual way—that is, the leaves were first rinsed in alcohol, then immersed in a 1 to 1,000 mercuric-chlorid solution. After rinsing in sterile water, small pieces of leaf tissue representing the various stages of infection were cut out and placed in the dishes, over which was poured corn-meal agar and synthetic agar. In both media there appeared after three to four days a delicate, white mycelial growth. Transfers were made to corn-meal agar, potato, etc. After about two weeks those transfers made on potato cylinders showed numerous, dark-brown fruiting bodies which were beginning to extrude cirri in the same way as the pycnidia on the leaves. Microscopic examination showed these spores to be of the same character as those found on the leaves placed in damp chambers.

From water suspensions of these spores corn-meal agar and beef-agar plates were made for the purpose of starting single-spore cultures. Under the microscope single spores were marked and, when germination had started, were transferred to corn-meal agar slants and potato cylinders. These transfers were used for the greater part of the inoculation experiments.

INOCULATION EXPERIMENTS

INOCULATIONS INTO KALMIA LATIFOLIA

The first inoculation tests were made on July 17, 1914, on twigs of *Kalmia latifolia*, which were broken off and placed in beakers and kept on a laboratory table. A suspension of the spores (from cirri extruded from pycnidia in corn-meal cultures 5 weeks old) was made in sterile water, and the leaves were sprayed on both sides with this suspension, and then placed under bell jars for three to four days. Some of the leaves which had been pricked with a sterile needle were held as controls. All of the leaves which had been pricked when inoculated showed signs of infection at the end of 11 to 15 days. The disease, however, did not progress rapidly, being confined to tiny brown spots scattered irregularly about over the leaf blade. This was undoubtedly due to the fact that the air in the laboratory was too dry for a rapid spread of the infection. The spots were typical of those observed on the original material, and cross sections showed the presence of the same delicate mycelium in the leaf tissues. Plates were poured, and pure cultures of the fungus inoculated were obtained.

On November 4, 1914, 12 plants of *K. latifolia* were inoculated by spraying on suspension of the spores from cultures 5 weeks old. Needle pricks were made in about half of the leaves inoculated. The plants were kept in the greenhouse under inoculating cages. On the same day six branches from a very large plant of *K. latifolia* were broken off, and placed in bottles standing in water, over which bell jars were placed, and kept in the laboratory after inoculation. Some of them were inoculated simply by smearing moistened spores over the leaf surfaces, but the majority were sprayed with the suspension of spores, after which needle pricks were made.

Two plants were inoculated by spraying with spores from a fungus belonging to the genus Phyllosticta, which had developed in the damp-chamber incubation. One other plant was inoculated by placing on both leaf surfaces spores of *Alternaria* sp., which had developed also in the damp chamber, and one was inoculated with spores of *Pestalozzia* sp., needle pricks having been made in both cases.

Two plants were sprayed with sterile water, pricked with sterile needles, then placed under bell jars, and kept as controls.

In this experiment the laurel plants were kept under the bell jars for 21 days, and during this time several new leaves had started; but the

disease progressed so rapidly owing to the very moist conditions that it soon reached these new leaves through the stem, when they promptly blighted. No disease appeared in the controls.

Those plants left under the cages in the greenhouse showed the first signs of the disease on November 18 in the form of numerous, small, brown spots on the upper surfaces of the leaves. On account of the moist air of the inoculating chambers, these spots soon coalesced, forming the typical brown, brittle areas (Pl. 14, A). On December 1 every plant and branch inoculated with the fungus was badly diseased; in fact, practically all of the leaves had either fallen or become brown, dry, and brittle. The stems in each case were also infected. Poured plates were made from these inoculated plants, and the fungus inoculated was isolated in pure culture from each of the inoculated plants.

The two plants inoculated with the spores of *Phyllosticta* sp. gave no positive results; nor did those inoculated with *Alternaria* sp. and *Pestalozzia* sp. The controls, both at the greenhouse and laboratory, remained healthy.

In the manner thus outlined inoculation experiments were conducted from time to time during a period of three years, inoculating in all about 50 plants of *K. latifolia* and several dozen cut branches, most of which gave positive results. One interesting fact observed in this connection was the effect of keeping the cut branches or plants under very moist conditions under bell jars. In many cases stimulation of growth resulted, and practically no injury was noticeable in any plant or branch. In one experiment (March 4, 1915) one of the control plants was allowed to remain standing in a very moist atmosphere under a bell jar from March 4 until April 14, when it showed several new leaves and appeared to be perfectly healthy. One of the leaf-inoculated plants was also left for this same length of time, when it showed on the stem the typical pycnidia from whch the yellow cirri were extruding (Pl. 15, B).

On January 7, 1916, experiments were started to determine whether stomatal infections are possible under ordinary conditions. One plant out of six inoculated on this date by smearing moistened spores on the uninjured lower surface of the leaves showed typical diseased areas. However, this one experiment is not considered of much value, as the infection may have started through some injury too small to be seen at the time of inoculation. A microscopic examination of numerous sections failed to show hyphæ penetrating the stomata. The leaves inoculated on this plant were new ones, and very delicate, and it can be readily seen that even handling them might result in minute injuries through which the hyphæ might enter.

The experiments conducted as just described seem to afford sufficient evidence as to the pathogenicity of the fungus isolated. It is a wound parasite, or at least not an exceedingly active parasite. However, once it gains entrance to the parenchyma of the plant it will kill living tissue and may involve entire branches or even the whole plant.

INOCULATIONS INTO OTHER PLANTS

The differences morphologically are not very great between the fungus here under consideration and certain species of Phomopsis which cause diseases of *Citrus* spp., eggplant, and apple. It was therefore thought advisable to carry out cross-inoculations.

Apple, orange, and lemon fruits and young, growing eggplants were inoculated with the fungus isolated from *K. latifolia*. The fruits and plants were kept under as favorable conditions for infection as possible, and the virulence of the cultures used in each experiment was tested out by inoculation into *K. latifolia*.

No infections resulted from any of the inoculations. In the experiment on apple bacterial softrots appeared, and in one case a fruit showed a spot just at the margin of one of the inoculation pricks which appeared at first somewhat like the Phomopsis-rot of apple described by Roberts (*8*, *9*). Microscopic examination revealed very large, brown hyphæ, and cultures gave no colonies of the mountain-laurel fungus.

Sufficient experiments were performed to indicate that the species of Phomopsis here described is not the same as *Phomopsis citri* Fawcett (*6*) which causes melanose and stem endrot of Citrus fruits (*4*, *7*). In addition to its inability to infect Citrus fruits, it differs morphologically from the fungus described by Fawcett (*4*), in that the pycnidia are not embedded deeply in the tissues of the host, and in no culture medium was there formation of Achyla-like branches with protrusion of the protoplasm from the ends, such as Fawcett describes.

It is not infectious to eggplant, and therefore not the same as *Phomopsis vexans* Harter (*10*), from which it differs also in pycnidial characters—for example, there is no beak either on the host or in culture media.

The negative results from the inoculations into apple distinguish it from *Phomopsis mali* Roberts (*9*), though morphologically it is very much like this species, with the exception that the spores are smaller, and often contain more than two oil drops.

EFFECT OF THE FUNGUS ON THE TISSUES

Microscopic examination of sections fixed in Carnoy's fluid and stained by Van Gieson's method shows that the diseased areas consist of a confused mass of disintegrated leaf tissue and mycelial cells. The fungus is seen to extend not far beyond these diseased areas; and this, together with the fact that the mycelium is extremely delicate and hyalin, makes it rather difficult to locate in the host tissues. Very young spots, sectioned horizontally and stained by special stains, such as Van Gieson's, give best results. In such sections the fungus may be seen ramifying through the intercellular spaces both in the palisade tissue and in the spongy mesophyll. The chloroplasts are greatly reduced in number for a considerable distance around the diseased area, and the nuclei

show signs of disintegration—that is, they lose chromatin and become more or less fragmented. The vascular portions of the leaf are not involved until such time as the toxic substances resulting from growth of the fungus have brought about disorganization of all the other tissue systems. When this occurs, a mass of disintegrated cells is seen interspersed with a few cells still retaining their original contour, but whose protoplasmic contents have been changed to dark-colored, more or less opaque coagulation products. In early stages the diseased cells may contain one to several brown, oily-appearing droplets, surrounded by either a few of the still intact chloroplasts and a fragmented nucleus, or dense coagulation products.

MODE OF ENTRANCE

From the experiments here reported it is apparent that the fungus is a wound parasite. As already noted in one experiment infection followed inoculation by spraying on a suspension of the spores in water, no needle-pricks being made. However, it was not possible in this case to demonstrate by microscopic sections that the hyphae had entered through the stomata, although a good many sections, both vertical and horizontal, were carefully examined. Slight wounds are almost always present, through which the hyphae might easily enter.

CULTURAL STUDIES

CULTURAL CHARACTERS

It was found that the fungus grows most rapidly, and produces the greatest abundance of pycnidia on steamed corn meal in flasks, and on steamed potato cylinders standing in test tubes in about 1 c. c. of distilled water. Transfers were made from these media to corn-meal agar; steamed string beans; beef agar; synthetic agar,[1] Kalmia agar; steamed leaves and twigs of *K. latifolia;* steamed corn meal plus litmus milk; litmus milk; litmus agar plus various sugars (saccharose, maltose, lactose, dextrose, galactose, mannit); plain litmus agar; corn-meal agar plus saccharose, maltose, and dextrose; Uschinsky's solution; Cohn's solution; Dunham's solution; peptonized beef bouillon; beef gelatin; milk; sterile distilled water; potato juice in fermentation tubes. On a majority of these media few or no pycnidia are formed. On steamed corn meal the spores germinate in 24 to 48 hours, forming a pure-white, delicate mycelium which soon covers the surface of the

[1] Prepared according to the formula furnished this laboratory by Mr. Frederick V. Rand: (*a*) 1,500 c. c. of distilled water and 36 gm. of agar. Cook in double boiler for one hour at 15 pounds, pressure. (*b*) 500 c. c. of distilled water, 200 gm. of dextrose, 40 gm. of peptone, 20 gm. of ammonium nitrate, 5 gm. of magnesium sulphate (crystals), 10 gm. of potassium nitrate, 5 gm. of potassium acid phosphate, and 0.2 gm. of sodium chlorid. Boil in a double boiler for 30 minutes, add agar, and cook for 5 minutes. Restore volume, titrate, cool to 60° C., and add whites of two eggs. Cook to coagulate eggs, filter, tube, and sterilize. This formula is modified from that given by Darwin and Hamilton. (DARWIN, FRANCIS, and ACTON, E. H., PRACTICAL PHYSIOLOGY OF PLANTS. ed. 3, p. 68. Cambridge, 1901.)

medium as a flat, furry layer. At the end of about 7 days a dark greenish tinge appears about the center or at the margins of the corn meal, followed by minute dark-green to black points, — the beginnings of the pycnidia. In about 15 to 20 days after inoculation the entire surface, sides, and often the bottom of the corn meal are covered with velvety, irregularly shaped, large fruiting bodies frequently having dark-green to black caps, and pure-white to light-gray and olive-green sides (Pl. 16, A, B). Sometimes when the pycnidia are very small (within 13 to 15 days after inoculation of the flask), the surface and sides of the medium will become yellow from the extrusion of the waxy coils and roundish masses of spores. At other times bodies are produced that are identical externally with the pycnidia, but which form no spores at all, even when held for months under various conditions. The spore masses vary in color, but are usually waxy yellow through amber to cream.

A greater abundance of mycelium is produced on steamed potato cylinders than on any other medium. Pycnidia frequently form within five days after inoculation, and vary in color from deep mouse-gray to dark ivy-green. When old, the seal-brown to black stroma is sharply marked with wavy white bands, and is tough and leathery in texture.

On corn-meal agar very little mycelium is produced. The pycnidia are rather small and often clustered, but form rapidly. Spores are produced promptly within the scattered groups of pycnidia, and but very few sterile bodies are formed.

Synthetic agar is a very good medium for the isolation of the fungus, as the mycelium grows quickly and abundantly, the medium at the same time inhibiting or preventing the growth of most intruding bacteria. The colonies are very beautiful, feathery in appearance, and of a color between pale dull gray and white. No pycnidia or sclerotia have ever been observed on this medium.

When litmus milk was added to steamed corn-meal flasks, the substratum was blued very decidedly at first, but at the end of 10 days was bleached almost throughout (cream), the control flasks remaining lilac.

In litmus milk a fair growth occurred, with coagulation of the milk at the end of five days, but the casein was not precipitated until the seventh day. The litmus was slightly reduced after 15 days, and at the end of 30 days was entirely reduced and the milk proteolyzed. In one tube proteolysis occurred in 13 days.

The growth of the fungus in Cohn's solution demonstrates its ability to obtain nitrogen from ammonium salts. There was no liquefaction of the medium when the fungus was grown in beef gelatin, and no pycnidia were formed.

GERMINATION TESTS

Practically all the transfers made during the work with this fungus have been germination tests, since the extruded spores were in almost every instance used to make the transfers. Spores freshly extruded germinate very promptly under favorable conditions—that is, within 24 hours they usually send out a germ tube from the blunter end, and rarely two, one from each end (fig. 1, A). Spores which are older and which have become dried of course germinate more slowly, though the age and condition of the spores and time required for germination vary in this direct ratio only up to a certain point. Long threads of spores which had stood in corn-meal flask cultures for 13 months and which had become very dry and brittle germinated about as soon as spores taken from similar cultures 6 to 8 weeks old. Steamed-potato cylinders and steamed corn meal in flasks have proved to be the best media for rapid germination.

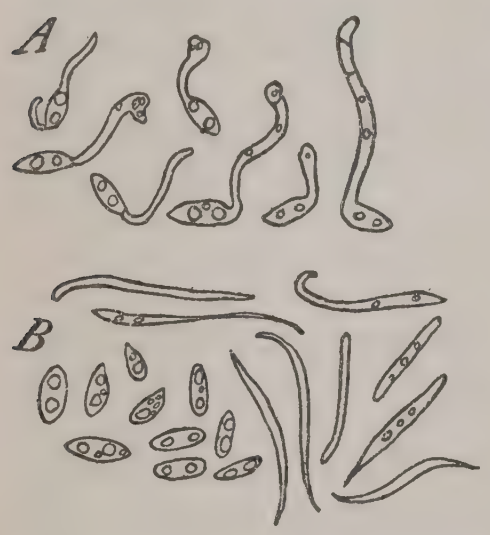

FIG. 1.—*Phomopsis kalmiae: A*, Germinating pycnospores; *B*, types of spores produced by the fungus. × 950.

Germination is very slow in distilled water, and only a small percentage of the spores introduced germinate at all. After 48 hours but very few spores are found which have produced a germ tube. Even after five days there is rarely any growth visible to the naked eye, but a microscopic examination shows a limited germination. Possibly 1 to 5 per cent of the spores are seen to have formed short germ tubes, very rarely branching. Very frequently the germinating spores showed peculiar swellings or knobs (appressoria?) at the end of the germ tube (fig. 1, A).

Several experiments were made by adding varying quantities of ether to sterile distilled water containing the spores, and very small amounts stimulated germination to a marked degree.

The germination of the sclerotia-like bodies, or sterile pycnidia, was also tested. With sterile forceps the upper portion of these erect aerial bodies was broken off and placed upon potato cylinders, corn-meal agar slants, etc. Growth occurred in practically all of the tests made with these bodies. Plates were also poured from corn-meal agar tubes, in which some of these sclerotial bodies had been placed, so that growth could be watched under the microscope. Plate 17, C, shows the appearance of a cross section of such a body after growth had started.

PHYSICAL AND BIOCHEMICAL CHARACTERS

REDUCTION OF NITRATES.—Cultures in Dunham's solution with potassium nitrate were tested after 5, 10, 15, and 20 days' growth by the starch-iodin method. No blue color reaction resulted, showing the absence of nitrites, as is also shown below under "Indol production."

INDOL PRODUCTION.—Tests for indol were made with cultures of varying age in Dunham's solution by means of the nitroso-indol reaction. No indol was produced at any time.

AMMONIA PRODUCTION.—Qualitative tests for ammonia were made with cultures grown in litmus milk, nutrient bouillon, and steamed corn meal by means of the calcium-hydroxid method. The results were positive in all cases, both in old and in young cultures.

VITALITY ON CULTURE MEDIA.—The fungus can live for a considerable period in flasks of corn-meal agar or on potato cylinders, but it loses its virulence rather quickly. Transfers on January 13, 1916, from corn-meal flasks made on January 29, 1915, grew promptly; but inoculations from these fresh transfers gave but a very small percentage of infections. In another experiment, 1 flask out of a series of 10 almost 2 years old sent out from its dried pycnidial or stromatic surface aerial hyphæ in about 10 days after the addition of freshly prepared sterile milk It was not possible to determine from what points these hyphæ were derived—that is, whether from the stroma or from the pycnidia or spores—since the growth began below the surface of the milk and was rather far advanced before it was observed. It is rather certain, however, that it was not from the old dried cirri, since 12 corn-meal flasks were inoculated from some of the dried extruded spores in this flask, previous to the addition of the milk, all with negative results.

TEMPERATURE RELATIONS.—The optimum temperature for growth lies between 20° and 25° C. Numerous tests were made on various media in all the compartments of a large thermostat. Germination will occur at much higher and lower temperatures, but the production of pycnidia and spores is in such cases very limited or absent. To test the effect of temperature on growth, spores were germinated on steamed-potato cylinders, corn-meal agar, etc., and then placed in the incubators at different temperatures, the range being from 1° to 37.5° C., where they were allowed to remain from two to five weeks. No growth occurred below 5°. No pycnidia were formed below 12°, and none above 28°. At 36° and 37.5° there was possibly a 10 per cent germination, but it was limited to the germ tube in most cases. The percentage of germination does not vary to any great extent between 18° and 35°, though the optimum point apparently lies very close to 25°.

MORPHOLOGY AND TAXONOMY

The hyphæ of the mountain laurel fungus are exceedingly fine, and it is located with difficulty in the tissues of its host. Nevertheless, this may be best accomplished by means of horizontal sections stained by Van Gieson's method.

The mycelium is septate, very fine, frequently branching, and hyalin. In the host the ends of the hyphæ are at times much swollen.

The pycnidia which form on leaves or stems of *K. latifolia* (Pl. 15, A, B) are very small compared to those on steamed white corn meal, and on steamed-potato cylinders. Those occurring on the leaves vary all the way from 75 to 500 μ in diameter. The majority, however, are small, usually about 250 μ in diameter. On steamed corn meal the pycnidia are from 0.5 to 5 mm. in diameter, the average being 2 mm. On the leaf of *K. latifolia* the color of the fruiting bodies is dark brown, almost identical with the color of the dead leaf tissue surrounding them. On culture media the color varies as stated elsewhere, being usually gray white or dark greenish olive to dark ivy-green. On the host the pycnidia are not deeply embedded, but are subepidermal and usually somewhat flattened and roundish (Pl. 15, A). There is no beak. No stroma is formed on leaves or stems. The outer wall of the pycnidium is rough and on sectioning is seen to be somewhat carbonaceous, of parenchymatous texture, and irregularly thickened, the thickest portion being toward the top (Pl. 15, D). The majority have but a single chamber.

The pycnidia on culture media are much larger than on the host. They are also much more irregular in form, but usually are roundish and somewhat flattened (Pl. 16). The carbonaceous wall is very much more pronounced than on the host. The outer surface is smooth and velvety in appearance. A cross-section of a pycnidium produced on steamed corn meal is shown in Plate 16, D, E. It is seen to be made up of very small pseudoparenchymatic cells, the majority of these cells being not over 10 μ in diameter. Usually the pycnidia on culture media are many-chambered. Frequently the chambering is limited to the outer portions of the pycnidium, and the cells are then coarser and show a more or less radial arrangement. Upon approaching the portion which is producing the spores, the parenchymatous structure is gradually lost, and only interlacing hyphæ appear, which are here very prominently nucleated (Pl. 16, E). The sporophores are borne upon a more or less carbonaceous mat of hyphæ.

Sterile pycnidia.—In the cultural tests conducted, it has been found that a large number of the bodies formed (usually upon a stroma) are sterile. The structure of these bodies is shown in Plate 17, A, B. It is identical with the internal central structure of a pycnidium which is producing spores only at its margins. In all the numerous sections made of these sterile bodies, small areas have been found at different points along the periphery, showing interlacing hyphæ prominently nucleated (Pl. 17, B), and which suggested to the writer that these bodies were really potential pycnidia, since areas similar to this are the spore-producing portions of the pycnidia. Not much time has been expended in determining this point, but the following experiment was repeated several times, and in each test spores were produced in 75 to 100 per cent of the bodies used in the experiments. From 6 to 25 (in one experiment 60) of these sterile structures were selected from the

stromatic surface of a culture on steamed corn meal. With sterile forceps these were freed from the stroma, placed in a tray, and bisected. One-half of each of these pycnidia-like bodies was at once fixed in Carnoy's fluid, and later embedded in paraffin. The corresponding half in each case was placed in a petri dish of corn-meal agar, or of corn meal. They were examined daily, and in all the experiments more than two-thirds, and in four experiments all of the bodies transferred produced spores in from 4 to 10 days. Rapid growth of mycelium always occurred in the transfers to dishes of steamed corn meal, but only about 5 per cent produced spores. In this medium the pycnidial bodies become covered with a white, dense mat of hyphæ and the surrounding medium is likewise overgrown with a thick stromatic layer. Such is not the case when corn-meal agar is used; only a very delicate fringe-like growth around the pycnidial bodies occurs, and their surfaces are not overgrown. This again goes to show that the character of the medium has much to do with the form and the fertility of these structures. As soon as spores were extruded, the bodies were fixed in Carnoy's fluid, embedded in paraffin, and sectioned to determine from what portion the spores had been produced. It was found that the portions of the sterile bodies showing the nucleated hyphæ were the points from which the spores originated (Pl. 17, A). It has been observed in the cultural studies made that these sterile bodies are produced only when the culture medium is of such character as to stimulate extremely rapid and abundant vegetative growth—that is, the stromatic surface becomes crowded, and possibly the oxygen or moisture requirements are inadequate. It might also be a question of food, since between the actively growing spore-potential cells lie a mass of dead cells resting upon a hard, dry stroma, frequently raised above the surface of the medium.

The sterility of these bodies might, too, be due to the production of toxic substances by the rapid mycelial growth. The central portion of the flasks where growth is more rapid is usually where the majority of the sterile bodies occur, the more scattered pycnidia on the margins producing spores in an almost constant ratio.

It should be noted that the object in sectioning one half of these sterile bodies when placing the other half in the culture medium was to determine absolutely that they were not then producing spores, and also to study the structure of each for comparison with the other half after it had fruited.

A few experiments were made to determine the effect of limited air supply upon the production of spores by these sterile pycnidia. About 100 of these bodies were bisected and arranged in rows on petri dishes of corn-meal agar. One half of each sterile pycnidium was left uncovered in the dish and the other half covered with sterilized slides or cover glasses. After five to seven days all of the bodies left uncovered in the dish were extruding masses of spores (Pl. 16, C), while those covered had produced no spores and only about one-third as much mycelium.

Two kinds of spores are found both in the pycnidia on the host and in culture media: An egg-shaped to spindle-shaped spore, and a much longer, slender, curved, sickle-shaped, or hooked spore (fig. 1, B). The former are designated by Diedicke (*3*) as α spores, and measure unstained 5.5 to 8.8 by 1.8 to 3.6 μ, the average size being 2 by 5.7 μ. Stained, 5 to 5.8 by 1.1 to 1.2 μ. They are hyalin, nonseptate, contain two to three oil drops (tested with Sudan III), and a deeply staining nucleus. They are borne on straight or slightly curved basidia (fig. 2, B) measuring 0.5 to 2 by 9 to 20 μ. The second form of spore which is found in the same pycnidium with the α form, Diedicke calls β spores and Shear (*5*) scolecospores. Reddick (*2*) regards them as free paraphyses. Shear states that—

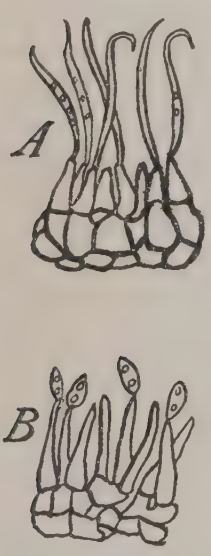

FIG. 2.—*Phomopsis kalmiae: A*, Scolecospores and basidia; *B*, the ordinary pycnospore and basidia. × 850.

> The term "scolecospore" is applied to these long, slender, crooked bodies tentatively to distinguish them from the ordinary pycnospores, because we regard them as reproductive organs, though they have not yet been found to germinate under the ordinary cultural conditions in which the other pycnospores germinate . . . true paraphyses are not normally abstricted and set free as these are, and occur typically only in connection with the asci, though the apparently sterile hyphae sometimes found intermixed with sporophores in pycnidia and acervuli, have been so designated by some mycologists. These slender, curved spores are evidently identical in character and origin with the so-called stylospores of Diaporthe as described by Nitschke [*1*], the other pycnospores being called spermatia by him.

The scolecospores are hyalin and contain one to several oil drops, often none, and most rarely a very pale-staining nucelus may be seen. They measure unstained 1.6 to 2.4 by 14 to 33.6 μ, average, 1.9 by 22 μ; stained, 0.6 to 0.8 by 13.2 to 23.1 μ. They are borne on short, tapering, occasionally almost pyriform basidia measuring 2.2 to 2.7 by 5.5 to 11.1 μ (fig. 2, A). The scolecospores occur in less number always than the other kind of spore and pycnidia have been found containing no scolecospores at all. No pycnidium has been found with only the scolecospores present, though there are chambers within the multichambered pycnidia which do contain only the scolecospores.

In some culture media a raised stroma is formed in which the pycnidia develop and in others the stroma is absent.

The spores are extruded in irregular droplike masses, cream, waxy-yellow or amber in color, or in definite threadlike form, measuring 10 to 25 mm. in length.

TECHNICAL DESCRIPTION OF THE FUNGUS

All attempts toward finding an ascogenous form of this fungus have been unsuccessful. Inasmuch as it differs from all other fungi so far described as infectious to *K. latifolia*, and as the genus in which it must

undoubtedly be placed from the characters given is Phomopsis, it is believed that the name "*Phomopsis kalmiae*" is a suitable one.

Phomopsis kalmiae, n. sp.

Pycnidiis in foliis et caulibus kalmiae latifoliae subglobosis, epidermide tectis, sparsis, sine stromate, fuscis, carbonaceis, ostiolatis, pleurumque unilocularibus. Pycnidiis in culturis subglobosis, vel saepe late ellipsoideis, non definite ostiolatis, plurilocularibus, sparsis, gregariis, vel in stromate aliquid denso, atro-viridibus, carbonaceis.

Sporulis ovatis, ellipsoideis, raro subfusoideis, continuis, hyalinis, typice 2-guttulatis, 1.8–3.6 × 5.5–8.8 μ. Basidiis filiformibus, pleurumque continuis, hyalinis, obtusis, 0.5–2 × 9–20 μ. Scolecosporulis filiformibus, attenuatis, fusoideis, rectis, vel leviter curvulis, vel hamatis, raro sigmoideis, saepe guttulatis, 1.6–2.4 × 14–33.6 μ. Basidiis brevibus, hyalinis, continuis, subulatis, 2.2–2.7 × 5.5–11.1 μ.

Pycnidia in leaves and stems scattered, subepidermal, brown, carbonaceous, usually unilocular, subglobose, without stroma, ostiolate. Pycnidia in culture media scattered, aggregate or in a rather thick stroma, subglobose to broadly elliptical, plurilocular, without definite ostiole.

Spores oval, ellipsoidal or rarely subfusoid, with two to several oil drops, continuous, hyalin, measuring 5.5 to 8.8 by 1.8 to 3.6 μ. Basidia filiform, tapering to blunt points, continuous, hyalin. Scolecospores filiform, attenuate, spindle-shaped and straight or slightly curved, or hooked and attenuate, occasionally delicately S-shaped, hyalin, continuous, and often contain one to several oil drops, measuring 1.6 to 2.4 by 14 to 33.6 μ. Basidia short, hyalin, continuous, subulate, 2.2 to 2.7 by 5.5 to 11.1 μ.

SUMMARY

The leafblight of *Kalmia latifolia* is characterized by a blight or dryrot involving large areas of either the leaf blade or the entire leaf. Later, it extends through the petioles into the stems and may eventually kill the entire plant. A fungus has been isolated whose parasitism has been demonstrated by successful inoculations into healthy plants. No published information upon this disease has been found, and the casual fungus is therefore described as a new species: *Phomopsis kalmiae.*

Pycnidia are readily produced on diseased leaves placed in damp chambers. Sclerotia-like bodies and pycnidia are produced in large numbers in most of the ordinary culture media. The sterile bodies are undoubtedly potential pycnidia as shown by the production of pycnospores after transplanting portions to fresh culture media.

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FRUIT-ROT, LEAF-SPOT, AND STEM-BLIGHT OF THE EGGPLANT CAUSED BY PHOMOPSIS VEXANS. *In* Jour. Agr. Research, v. 2, no. 5, p. 331–338, 1 fig., pl. 26–30. Literature cited, p. 338.

PLATE 14

A.—Twig of *Kalmia latifolia* showing late stage of infection with *Phomopsis kalmiae*. Inoculation of March 4, 1915. Slightly reduced.

B.—A leaf of *K. latifolia* in an incipient stage of infection. Note the lighter green area surrounding the diseased portions. Inoculation of January 6, 1916. About natural size.

C.—Plant of *K. latifolia* showing the intermediate stage of the disease. Reduced one-half. Photographed by Mr. James F. Brewer.

A

B

C

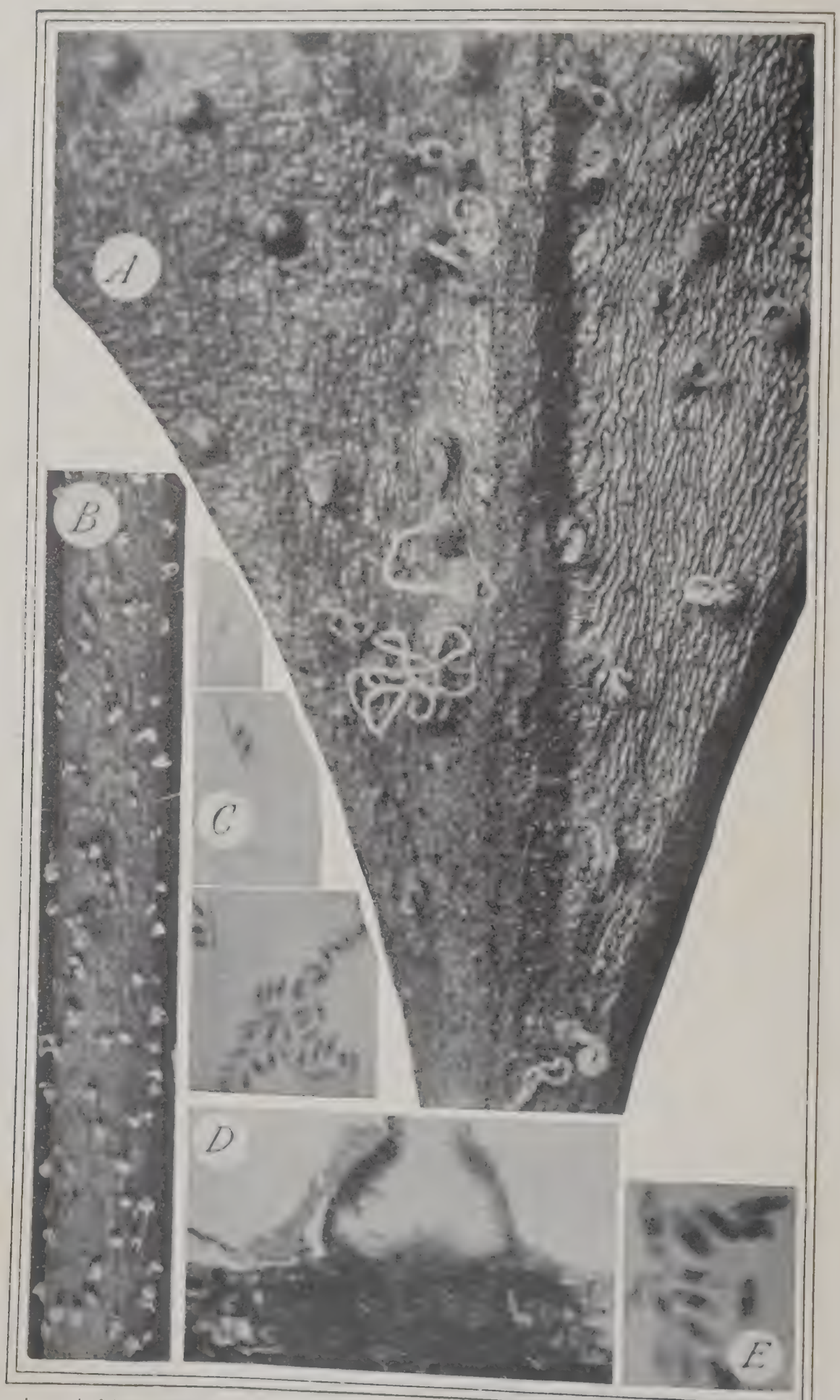
A
B
C
D
E

PLATE 15

A.—A leaf of *Kalmia latifolia* enlarged 13 times to show the character of the pycnidia of the fungus on its host. Photographed by Mr. James F. Brewer.

B.—A stem of *Kalmia latifolia* 40 days after inoculation in leaves only. The plant was kept under very moist conditions, which favored the production of pycnidia. Spores in the form of cirri may be seen extruding from the pycnidia. ×5.

C.—Photomicrograph showing both kinds of spores of *Phomopsis kalmiae*, from culture on corn meal. Note the relative staining properties of the two types. A 12 ocular and a 4 objective were used. The stain was Delafield's hematoxylin and eosin. × 695. Photomicrograph by the writer.

D.—Section through a pycnidium of *Phomopsis kalmiae* on leaf of *K. latifolia*. Note the superficial character of the pycnidium and its ostiole, from which the spores lying to left have been extruded. × 92.

E.—The ordinary type of spore of *Phomopsis kalmiae* more highly magnified (12 ocular and 2 mm. oil-immersion lenses). Photomicrograph by the writer. × 1,200.

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PLATE 16

Phomopsis kalmiae:

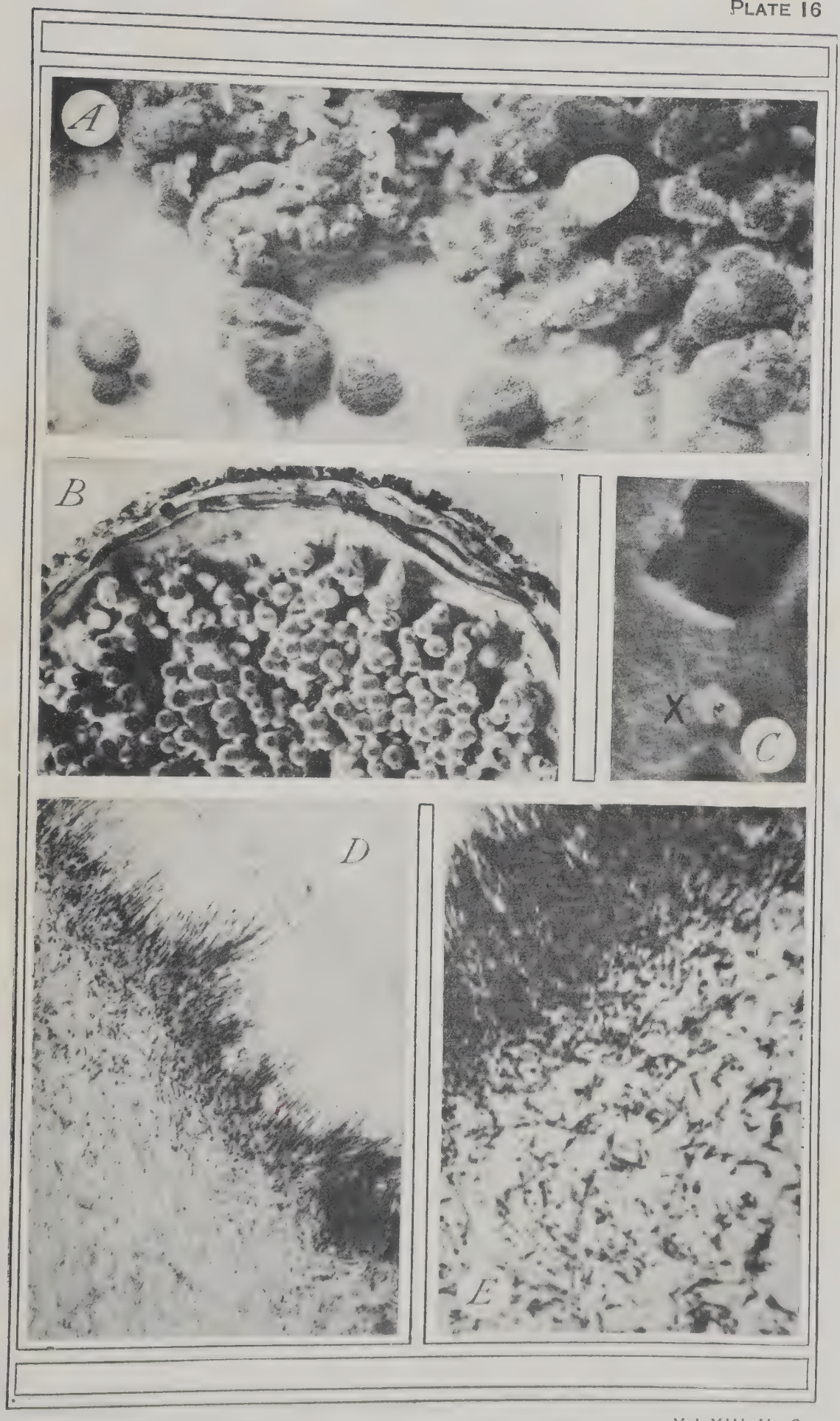
A.—An 18-day-old culture on steamed corn meal enlarged about 5 times. Note the extruding spore masses. Photographed by Mr. James F. Brewer. × 6.

B.—A 15-day-old culture on same medium, natural size. Later, the peripheral pycnidia extruded cirri freely, but the central ones did not. Photographed by Mr. James F. Brewer. × 1.1.

C.—Portion of corn-meal agar plate on which were sown three bisected sterile pycnidia. One half of each pycnidium was covered with sterile glass, and no spores were produced. The section left uncovered produced spores within five days, as here shown at X. Slightly reduced.

D.—Section through a pycnidium, showing the sporophores and the nucleated hyphæ just below them. × 175. Photomicrograph by the writer.

E.—A portion of same section shown in figure A more highly magnified; about 500. Photomicrograph by the writer.


A
B
X
C
D
E

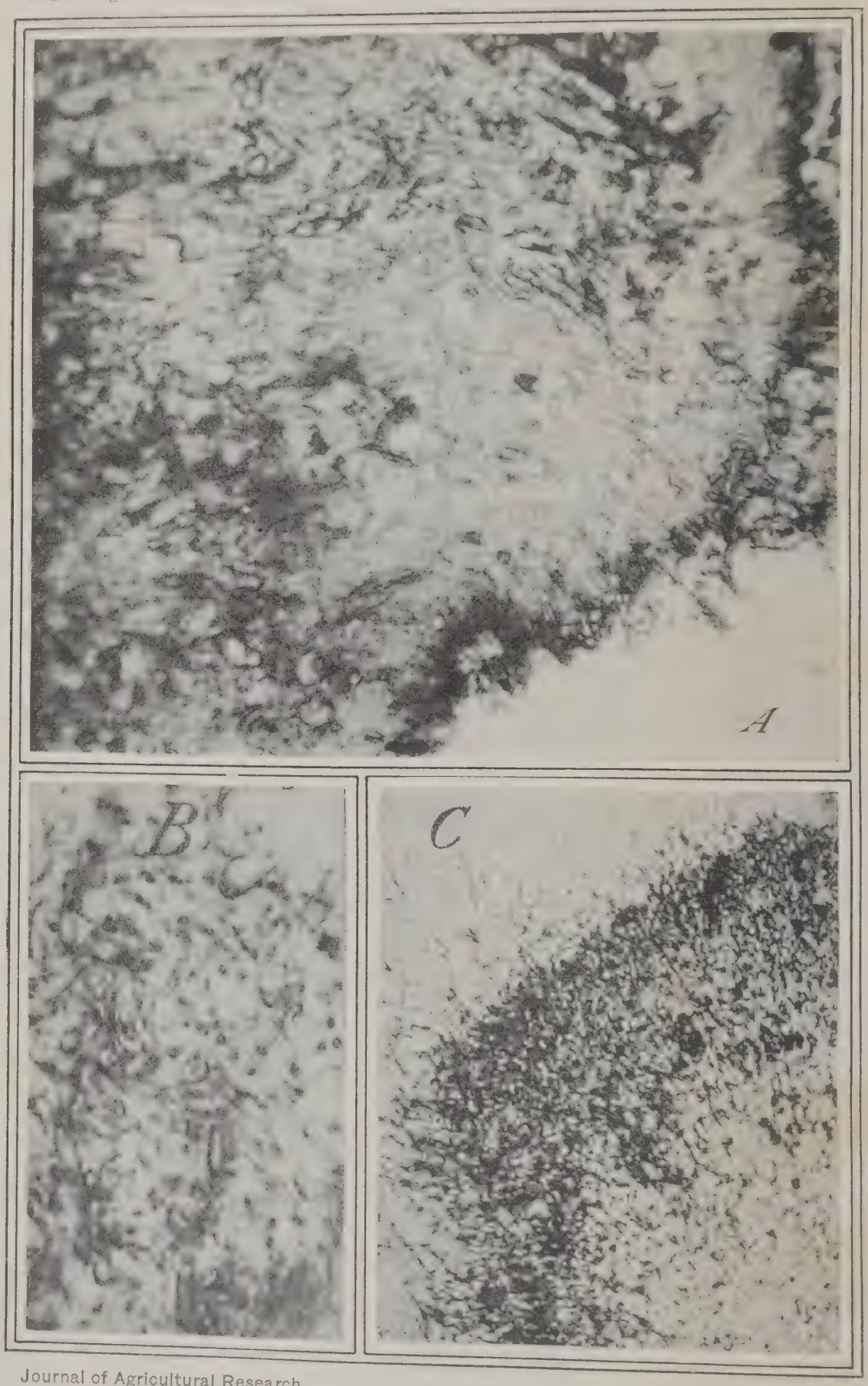

A
B
C

PLATE 17

Phomopsis kalmiae:

A.—Section through a sterile pycnidium, showing an area containing nucleated hyphæ. From such areas spores arise when the pycnidium is transferred to a suitable medium. × 200.

B.—Central portion of figure 1 more highly magnified. × 625.

C.—Section through a sterile pycnidium, showing growth beginning at the margins after it had been transferred to a more suitable medium. × 110.

Photomicrographs by the writer.

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RELATION BETWEEN BIOLOGICAL ACTIVITIES IN THE PRESENCE OF VARIOUS SALTS AND THE CONCENTRATION OF THE SOIL SOLUTION IN DIFFERENT CLASSES OF SOIL [1]

By C. E. MILLAR [2]

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INTRODUCTION

Experiments dealing with the concentration of the soil solution in soils of different classes when treated with various salts have been in progress in this laboratory for some time, and it has now become very desirable to know the relation between the biological activities and the composition and concentration of the soil solution under these conditions.

Several workers have shown that certain salts occurring naturally in many soils and often added as fertilizers and amendments have pronounced effects on the bacterial flora present. The causes of these effects have not been entirely determined, it being assumed that both toxicity and osmotic pressure are operative.

HISTORICAL REVIEW

No attempt will be made to give a complete résumé of the literature on toxicity, but the writer wishes to call attention to a few points brought out by various investigators.

A review of the available literature on the toxicity of salts for higher and lower plant life shows considerable disagreement in the results obtained by different investigators. It is suggested by Greaves [3] that in many cases this may be explained by the unknown variation in the actual amounts of salts added, and he proposes to remedy the deficiency by analyzing the solutions used to supply the salts and correcting for the variations found.

Greaves [3] found that calcium nitrate, magnesium nitrate, calcium chlorid, sodium sulphate, and potassium sulphate were toxic to the

[1] Publication authorized by Dean R. S. Shaw, Director of the Michigan Agricultural Experiment Station.

[2] The writer wishes to express his gratitude to Dr. M. M. McCool, of the Michigan Experiment Station, for many helpful suggestions during the completion of these experiments and preparation of the manuscript.

[3] GREAVES, J. E. THE INFLUENCE OF SALTS ON THE BACTERIAL ACTIVITIES OF THE SOIL. *In* Soil Science, v. 2, no. 5, p. 443–480, 4 fig. 1916.

ammonifiers in very low concentration (78×10^{-3} mol. per 100 gm. of soil) and all the other salts used, with the exception of calcium carbonate and manganous carbonate, became toxic in some of the concentrations tested. He states (p. 474) that—

> The increased osmotic pressure exerted by the salts added to a soil plays an important part in the retarding of the bacterial activity, it is not the only nor probably the main influence. The main influence is likely to be a physiological one due to the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it cannot function properly.

Osmotic pressure as the causal factor is also suggested by the work of Harris,[1] who found that the germination of different seeds was first retarded by the salts studied when the soils contained a small amount of moisture. With most of the salts the highest germination was in the wettest sand, while with sodium chlorid the medium moisture gave the highest germination.

He also states that (p. 44)—

> In a general way salts with low molecular weights are more toxic than those having a higher molecular weight, but there are so many exceptions that this can not be considered a general law holding for all salts.

That the soil plays an important rôle in the toxic action of salts was shown by Harris,[1] who points out (p. 26) that—

> Plants were able to endure much stronger chlorids and nitrates in solution culture than in the soil, while the carbonates retarded growth more in the solution than in the loam, but not as much as in the sand.

The antagonism of salts was found to be less in soils than in solutions. He also concludes that only about one-half as much alkali is required to prohibit the growth of crops in sand as in loam soil. Sodium carbonate was more toxic in sand than sodium chlorid, while with loam the reverse was true.

That soils of different chemical and physical properties influence differently the effect of added substances on the lower organisms is shown by the work of Lipman and Burgess[3] when they found that the order of nitrifiability of various nitrogenous substances depends largely on the soil used.

Further evidence of the importance of the interaction of the soil with the salts added is brought out by Headley, Curtis, and Scofield[4] in determining the relative toxicity of sodium carbonate, sodium bicarbonate, sodium chlorid, and sodium sulphate for wheat. They not only

[1] Harris, F. S. Effect of alkali salts in soils on the germination and growth of crops. *In* Jour. Agr. Research, v. 5, no. 1, p. 1–53, 48 fig. 1915. Literature cited, p. 52–53.

[3] Lipman, C. B., and Burgess, P. S. The determination of availability of nitrogenous fertilizers in various California soil types by their nitrifiability. Cal. Agr. Exp. Sta. Bul. 260, p. 103–127. 1915.

[4] Headley, F. B., Curtis, E. W., and Scofield, C. S. Effect on plant growth of sodium salts in the soil. *In* Jour. Agr. Research, v. 6, no. 22, p. 857–869, 8 fig. 1916.

measured the amount of the salt added but at the completion of the period of growth extracted the soil with water and determined the quantity of salt removable. They arrived at the following conclusion (p. 869):

> The limit of tolerance of crop plants to the salt in the soil is determined by the quantity of salt that can be recovered from the soil rather than by the quantity added to the soil.

The toxicity of sodium carbonate was much greater in beach sand than in loam soil, and the loss of carbonate was much less in the sand than in the loam.

Bouyoucos and McCool [1] studied more in detail the action of salts in soils and found that equal amounts of various salts added to different soils result in widely different concentrations of the soil solution and suggest that there is probably considerable change in the chemical composition of the resulting soil solution.

More definite information regarding the difference in end products formed when salts come in contact with different soils is furnished by McCool and Wheeting,[2] who show that the amounts of water-soluble calcium, iron, and magnesium in soils through which various salts have diffused are quite different and that the quantities of these elements in the layers of soil at various distances from the salt deposit are variable.

This interaction of soil and salt has seemingly been overlooked by some investigators in accounting for the toxic action of salts; and it was with the hope of obtaining more evidence as to whether osmotic pressure, the toxic properties of the salt itself, or the products of the interaction of the soil and salt was responsible for the effects noted that the following experiments were conducted.

EXPERIMENTAL WORK.

Three soils were used in this work: A Coloma sand, a Miami sandy loam, and a Clyde clay loam. The soils were brought to the laboratory, dried, passed through a 20-mesh sieve, mixed thoroughly, and stored in the dark. As needed, 100-gm. samples were weighed into sterile, covered tumblers and thoroughly mixed with 2 gm. of finely ground dried blood. The salts were applied in solution. The most concentrated solution was made by weighing out the correct amount of the salt and dissolving it in distilled water. The solution of next lower concentration was made by drawing off a portion of the first solution and making it up to the required volume by weight. The third solution was made from the second in a similar manner. The solutions were

[1] BOUYOUCOS, G. J., and MCCOOL, M. M. THE FREEZING-POINT METHOD AS A MEANS OF MEASURING THE CONCENTRATION OF THE SOIL SOLUTION DIRECTLY IN THE SOIL. Mich. Agr. Exp. Sta. Tech. Bul. 24, p. 592–631, 2 fig. 1916.

[2] MCCOOL, M. M., and WHEETING, L. C. MOVEMENT OF SOLUBLE SALTS THROUGH SOILS. *In* Jour. Agr. Research, v. 11, no. 11, p. 531–547, 5 fig. 1917.

sterilized, a small quantity was poured into a sterile container, and the desired amount was drawn off in a sterile pipette graduated to 0.01 c. c. The solution was mixed with enough sterile water to make the soil up to optimum moisture content, and the liquid was thoroughly mixed through the soil with a sterile spatula. The samples were incubated for four days at 29° to 30° C. and then distilled from copper flasks with magnesium oxid in the usual way. The data presented represent the average of three or more determinations which agree within 12 per cent and with a few exceptions within 10 per cent.

The freezing-point determinations were made by mixing 25 gm. of soil with a proportional amount of solution, after which the mixture was placed in a freezing tube and the freezing point determined with a Beckman thermometer. The data given represent the average of two determinations.

The following tables (I–VII) show the amount of the salt added, the nitrogen produced from 2 gm. of dried blood, the depression of the freezing point of the soil solution, and the corresponding osmotic pressure.

While working with the sandy loam, it became apparent to the writer that the increments by which the amounts of the salt used in the various tests increased were much smaller than necessary to bring out the points desired, and consequently some of the concentrations were omitted in the clay-loam series.

TABLE I.—*Ammonification of dried blood in sandy loam and clay loam with varying percentages of magnesium sulphate*

Salt in air-dry soil.	Sandy loam.			Clay loam.		
	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.
Per cent.	*Mgm.*	*°C.*	*Atmospheres.*	*Mgm.*	*°C.*	*Atmospheres.*
0.000	75.70	0.285	3.435	52.10	0.276	3.327
.001	69.67	.289	3.483	56.36	.288	3.471
.002	71.91	.275	3.315	.....	.....	.....
.008	76.12	.280	3.375	58.60	.290	3.496
.016	77.38	.308	3.712	56.36	.315	3.797
.032	76.54	.280	3.375	57.20	.288	3.471
.064	68.97	.330	3.978	53.84	.308	3.712
.100	67.57	.333	4.014	52.02	.303	3.652
.200	61.87	.403	4.857	.....	.....	.....
.300	62.67	.462	5.567	49.64	.373	4.495
.400	68.41	.530	6.386	.....	.....	.....
.500	69.25	.568	6.843	44.87	.455	5.483
.600	61.41	.610	7.349	.....	.....	.....
.700	54.82	.725	8.732	48.52	.483	5.820

The data in Table I show that for sandy loam the addition of 0.001 and 0.002 per cent of the salt resulted in a slightly lower ammonification than the untreated sample. The differences, however, are within the limits of experimental error. Concentrations of 0.008, 0.016, and 0.032

per cent of the salt gave results practically identical with those of the untreated soil; but with an addition of 0.064 per cent a marked decrease in ammonia production resulted. With a higher percentage of the salt the results show irregularities due to experimental errors, but they also show a general tendency to depress ammonification still further. When 0.7 per cent of the salt was added, a very great decrease resulted in the amount of ammonia produced.

A tendency to increase the osmotic pressure in the sandy-loam series is noticeable with an addition of 0.016 per cent of the salt. This tendency, however, is more pronounced at 0.064 per cent; and thereafter the osmotic pressure rises steadily with no irregularities.

The results with clay loam show a small but persistent stimulation of the ammonifying organisms until a concentration of 0.064 per cent of the salt is reached, when the production of ammonia drops back to practically that of the untreated soil.

There is very little further decrease in the amount of ammonia produced with a greater application of the salt, except in the case of 0.500 per cent. Since, however, the production with 0.700 per cent rises to practically that of the untreated soil, it seems probable that the reduction with 0.500 per cent of the salt is an experimental error. There is no point, therefore, at which it can be said that a marked toxicity to ammonification occurs.

It is interesting to note that there is no marked increase in the osmotic pressure of the soil solution until an addition of 0.300 per cent of the salt has been made, which is somewhat more than the amount present when ammonification was reduced to normal.

TABLE II.—*Ammonification of dried blood in sandy loam and clay loam with varying percentages of calcium nitrate*

Salt in air-dry soil.	Sandy loam.			Clay loam.		
	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.
Per cent.	*Mgm.*	*°C.*	*Atmospheres.*	*Mgm.*	*°C.*	*Atmospheres.*
0.000	75.70	0.285	3.435	52.10	0.276	3.327
.001	76.68	.263	3.170	55.52	.275	3.315
.002	75.56	.280	3.375	.....	.....	.....
.008	77.38	.252	3.038	55.24	.285	3.435
.016	74.72	.277	3.339	51.88	.295	3.546
.032	75.70	.307	3.700	49.92	.290	3.496
.064	73.59	.364	4.387	55.66	.330	3.978
.100	71.35	.394	4.748	53.70	.393	4.736
.200	64.49	.523	6.302	.....	.....	.....
.300	58.18	.685	8.251	48.24	.568	6.843
.400	56.08	.790	9.514	.....	.....	.....
.500	54.82	.930	11.20	49.36	.683	8.227
.600	50.89	1.060	12.76	.....	.....	.....
.700	48.04	1.152	13.864	47.54	.873	10.516

A consideration of Table II shows there is no apparent stimulation or depression of ammonification in sandy loam until a concentration of 0.200 per cent of calcium nitrate is reached, when a marked depression occurs, and is followed by further slight depressions with each succeeding addition of the salt. The freezing-point determinations show a well-defined increase in the osmotic pressure of the soil solution when 0.032 per cent of the salt is added, with a further increase with each succeeding increase in the amount of calcium nitrate added.

With clay loam there is apparently no stimulation of the bacterial activities such as occurred in the case of magnesium sulphate. The two series are similar, however, in that there is no point at which the salt exhibits a marked toxicity such as is seen in the case of sandy loam.

The osmotic pressure of the soil solution is decidedly increased when 0.064 per cent of calcium nitrate is present, and steadily rises as more of the salt is added.

TABLE III.—*Ammonification of dried blood in sandy loam and clay loam with varying percentages of calcium chlorid*

Salt in air-dry soil.	Sandy loam.			Clay loam.		
	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.
Per cent.	*Mgm.*	*°C.*	*Atmospheres.*	*Mgm.*	*°C.*	*Atmospheres.*
0.000	75.70	0.285	3.435	52.10	0.276	3.327
.001	78.22	.280	3.375	46.13	.280	3.375
.002	79.76	.290	3.496	.....	.....	.....
.008	80.18	.280	3.375	43.19	.300	3.616
.016	75.84	.316	3.809	42.21	.315	3.797
.032	73.59	.353	4.255	50.90	.320	3.857
.064	66.17	.450	5.423	55.10	.430	5.182
.100	60.43	.523	6.302	49.22	.470	5.664
.200	59.72	.708	8.528	.....	.....	.....
.300	59.02	1.068	12.856	47.12	.810	9.755
.400	59.86	1.385	16.660	.....	.....	.....
.500	52.30	1.620	19.48	44.31	1.163	13.996
.600	45.99	1.885	22.660	.....	.....	.....
.700	43.05	.....	.....	45.29	1.455	17.500

When calcium chlorid is added to sandy loam, we again see (Table III) an apparent slight stimulation of ammonification with 0.001, 0.002, and 0.008 per cent of the salt; but when 0.016 per cent is added, the process is reduced to normal; and with 0.064 per cent there occurs a marked depression. With further addition of the salt, ammonification is depressed as a whole, but the results are irregular.

The freezing points of the soil show that an increase of osmotic pressure occurred when 0.016 per cent of calcium chlorid was added, which agrees with the point brought out in Table II—namely, that an increase in osmotic pressure of the soil solution is observed with amounts of salts

which do not depress ammonification. It is rather striking in this series, however, that when an increase in osmotic pressure occurs the ammonia produced drops to that of the untreated soil.

The data with clay loam are exceedingly interesting. There appears at first to be a slight depression of ammonia production, the depression increasing until with 0.016 per cent of calcium chlorid it reaches almost 9 mgm. of nitrogen, or 19 per cent, considering the production in the untreated soil as 100 per cent. With 0.032 per cent of salt ammonification returns to normal and remains so until 0.300 per cent of salt is present, when it is slightly depressed again and remains so with but small increases of depression throughout the series. In no other concentration in the series, however, is the ammonia production reduced to quite so low a point as with 0.008 and 0.016 per cent of the salt.

The osmotic pressure of the soil solution shows a sharp increase with addition of 0.064 per cent of calcium chlorid, and thereafter there is a further increase with each addition of the salt. It is noteworthy, however, that ammonification returns to normal before there is any appreciable increase in the concentration of the soil solution.

TABLE IV.—*Ammonification of dried blood in sandy loam and clay loam with varying percentages of potassium chlorid*

Salt in air-dry soil.	Sandy loam.			Clay loam.		
	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.
Per cent.	*Mgm.*	*°C.*	*Atmospheres.*	*Mgm.*	*°C.*	*Atmospheres.*
0.000	75.70	0.285	3.435	52.10	0.276	3.327
.001	71.63	.280	3.375	50.08	.260	3.134
.002	73.87	.273	3.291	.....	.....	.....
.008	73.45	.298	3.592	45.85	.276	3.327
.016	69.53	.325	3.917	49.08	.305	3.676
.032	67.99	.373	4.495	48.38	.302	3.640
.064	60.43	.440	5.302	50.48	.336	4.050
.100	57.20	.538	6.482	45.71	.421	5.074
.200	54.26	.858	10.336	.....	.....	.....
.300	63.65	1.118	10.768	38.29	.765	9.214
.400	57.06	1.410	16.960	.....	.....	.....
.500	52.44	1.723	20.716	38.43	1.093	13.156
.600	44.03	.....	.....	.....	.....	.....
.700	33.95	.....	.....	35.07	1.455	17.500

The data in Table IV show a well-defined decrease in ammonification when 0.064 per cent of potassium chlorid is added to sandy loam; however, a decided increase in the osmotic pressure of the soil solution is noticeable when only one-fourth of this amount of the salt is added. This agrees closely with the results obtained when calcium chlorid and calcium nitrate are applied to this soil.

With clay loam the results are quite uniform until 0.300 per cent of salt is added, when there occurs a sharp decrease in the amount of ammonia produced. This is the only salt tested which gives results of this kind with clay loam. With the other salts the decrease in ammonification is gradual, no special point being observed where a marked decrease in ammonification occurs. As in the results with sandy loam, an increase in the osmotic pressure of the soil solution is observed somewhat before any decrease in bacterial activities are evident.

GENERAL DISCUSSION

The data presented bring out, first of all, that there is a decided difference in the effect on ammonification of the salts when applied to different soils. With every salt tested there was found with sandy loam a point at which marked depression of the ammonifying power occurred, while with clay loam such a point was found for potassium chlorid only. Whether this difference is due to a difference in the flora present or to the physical or chemical properties of the soil is undetermined. In the light of the work of Bouyoucos and McCool [1] and McCool and Wheeting,[2] however, there is unquestionably a difference in the composition of the soil solution. The difference in the behavior of these soils emphasizes the importance of much thorough investigation of the problem of toxicity of salts in soils.

Another point of interest is the lack of regularity in the results after a point is reached where ammonification is markedly depressed. It might be expected, whether the depression is due to toxicity or osmotic pressure, that from this point every addition of salt would cause an ever-increasing amount of depression. In no case, however, is this found to be true. Where there is a consistent decrease in ammonia production after this critical point is passed, such as occurs with calcium nitrate, the decreases are small. In many cases there are fluctuations, such as occur with potassium chlorid and magnesium sulphate, which are generally ascribed to experimental error. Again there are cases where further additions of salt fail to exert any further depressing effect on the ammonifying organisms, even though the amount of salt added may be increased four times, as is seen in the tests with calcium chlorid. It seems when such results as referred to above are obtained that there must be some fundamental cause for the variation, a possible explanation being complex chemical interchanges between the salts and the soil constituents, the end products depending on the amount of salt present. That some such factor is operative is made more evident by such results as those

[1] Bouyoucos, G. J., and McCool, M. M. Op. cit.
[2] McCool, M. M., and Wheeting, L. C. Op. cit.

obtained when calcium chlorid is added to clay loam; and in fact all the data from clay loam indicate the operation of some influence as yet not understood.

That the soil used has an important bearing on the results to be expected is made further evident by a comparison of the amounts of the different salts needed to bring about changes in the osmotic pressure of the soil solution and also in the depression of ammonification. A summary of results showing these points is given in Table V.

TABLE V.—*Percentage of salts and the resulting osmotic pressures at which depression of ammonification and increase in osmotic pressure of the soil solution are observed.*

Salt used.	Sandy loam.				Clay loam.			
	Depression of ammonification.		Increase in osmotic pressure.		Depression of ammonification.		Increase in osmotic pressure.	
	Salt.	Osmotic pressure.	Salt.	Osmotic pressure.	Salt.	Osmotic pressure.	Salt.	Osmotic pressure.
	Per cent.	*Atmospheres.*	*Per cent.*	*Atmospheres.*	*Per cent.*	*Atmospheres.*	*Per cent.*	*Atmospheres.*
Magnesium sulphate	0.064	3.978	0.016	3.712	.....	.....	.....	.....
Calcium nitrate	.200	6.302	.032	3.700	.....	.....	0.064	3.978
Calcium chlorid	.064	5.423	.016	3.809	.....	.....	.064	5.182
Potassium chlorid	.064	5.302	.016	3.917	0.300	9.214	.064	4.050

These data show that four times as much of the salt is required to produce a noticeable increase in the osmotic pressure of the soil solution in clay loam as in sandy loam when calcium chlorid and potassium chlorid are added. In the case of calcium nitrate only twice as much is required, while with magnesium sulphate 19 times as much is necessary. It is also noticeable that in sandy loam depression of ammonification occurs with the same amount of each salt except where calcium nitrate is used, when a greater amount is also needed to increase the osmotic pressure of the soil solution.

There is considerable variation in the osmotic pressure at which ammonification is depressed in sandy loam. The osmotic pressure of the soil solution in clay loam when ammonification is decreased by potassium chlorid is much greater than the osmotic pressure in sandy loam when depression occurs as the result of additions of any salt.

It appears from these data that the decrease in ammonification resulting from the addition of the salts used is not due to a change in osmotic pressure and that the soil itself is a very potent factor in determining the effect.

In order to judge more accurately the part played by osmotic pressure in modifying the action of ammonifying organisms a series was run with potassium chlorid in sand. The results are given in Table VI.

TABLE VI.—*Ammonification of dried blood in sand with varying percentages of potassium chlorid*

Salt in air-dry soil.	Sand.		
	Nitrogen produced.	Depression of freezing point.	Osmotic pressure.
Per cent.	*Mgm.*	*°C.*	*Atmospheres.*
0.000	32.64	1.157	13.924
.001	33.34	1.109	13.348
.002	31.80	1.120	13.48
.004	32.36	1.137	13.684
.008	24.73	1.101	13.372
.016	29.14	1.086	13.072
.032	28.44	1.160	13.96
.064	23.82	1.587	19.084
.100	27.88	1.837	22.084
.200	16.53	2.746	32.962
.300	10.09	.....	.....
.400	6.44	.....	.....
.500	5.88	.....	.....

These results show ammonification proceeding normally when the osmotic pressure of the soil solution is from 13 to 14 atmospheres. This is twice the osmotic pressure in sandy loam when calcium nitrate depressed ammonification and three times that when magnesium sulphate caused a decrease in ammonia production. It seems justifiable from the data presented to conclude that osmotic pressure is not the determining factor in the reduction of ammonification by the salts used.

One point should be mentioned in this connection. Neither in this work nor in the results of other workers reviewed by the writer have the total osmotic pressures of the soil solution been reported at the time the ammonia determinations were made. It is possible that the pressure due to the products of bacterial action plus that due to the salt added may equal a constant.

TABLE VII.—*Effect of dried blood on the osmotic pressure of the soil solution*

Kind of soil.	Amount of soil.	Amount of dried blood.	Freezing-point depression.	Osmotic pressure.	Difference in osmotic pressure due to dried blood.
	Gm.	*Gm.*	*°C.*	*Atmospheres.*	*Atmospheres.*
Sand	100	0.0	0.160	1.930	.....
Do	100	1.0	.267	3.218	1.288
Do	100	2.0	1.157	13.924	11.994
Sandy loam	100	.0	.135	1.518	.....
Do	100	1.0	.190	2.291	.773
Do	160	2.0	.285	3.435	1.917
Clay loam	100	.0	.160	1.930	.....
Do	100	1.0	.195	2.351	.421
Do	100	2.0	.276	3.327	1.397

One point which, so far as the writer is informed, has been omitted in toxicity work is the increase of osmotic pressure due to the addition of dried blood. Table VII shows the increase in the osmotic pressure of the soil solution of the soils used in the above experiments due to the addition of various amounts of dried blood.

It is very evident that the amount of blood used in ammonifying tests has a great influence on the concentration of the soil solution and that the increase in osmotic pressure is quite different with various classes of soils. Just what effect the addition of varying amounts of such material will have on the ammonifying power of the soil is yet to be determined. It seems improbable that results obtained under such abnormal conditions will represent the power of soils to ammonify nitrogenous materials under field conditions where much lighter applications are made.

CONCLUSIONS

Since the osmotic pressure at which ammonification of dried blood is depressed in sandy loam by the addition of various salts is different for each salt tested, it seems improbable that osmotic pressure is the governing factor. This conclusion is further strengthened by the observation that ammonification proceeds unimpaired in sand where the osmotic pressure of the soil solution is between 13 and 14 atmospheres, while in sandy loam the process is depressed when the osmotic pressure reaches 4 to 6 atmospheres and in clay loam at a pressure of about 9 atmospheres.

The effect of various salts on ammonification is apparently modified very materially by the nature of the soil used. Thus, for the four salts studied, each gave a definite point where the ammonification of dried blood in sandy loam was depressed, while only one salt gave such a point with clay loam. The cause of such variations is yet to be investigated, but it seems possible that the chemical reaction between the salt added and the soil constituents may play some part.

The addition of the amount of dried blood usually used in ammonification work has a very appreciable effect on the osmotic pressure of the soil solution, the increase varying with the class of soil used.

The effect of various salts on the ammonification of nitrogenous materials in soils offers the opportunity for much thorough investigation, one of the chief needs being improved methods.

BACTERIAL FLORA OF ROQUEFORT CHEESE

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INTRODUCTION

It is well known that various kinds of hard cheese, such as Cheddar and Emmental, or Swiss, depend on a suitable bacterial flora for their normal development. The two types of cheese mentioned have been studied extensively by a number of investigators. It has been shown that in the manufacture of Cheddar cheese there must be a rapid development of the lactic-acid bacterium *Streptococcus lacticus*, and that during the ripening a development of other forms of cocci and lactic bacteria of the *bulgaricum*[1] type is necessary to produce the typical Cheddar flavor (*4*).[2] In Swiss-cheese manufacture the *Bacterium bulgaricum* is added, either in pure culture, as a starter, or unwittingly, with the rennet. What other microorganisms are responsible for the characteristic sweetish flavor, and for the development of the "eyes"; has not yet been established, but at any rate the ripening is due to the growth of bacteria, in distinction from the mold-ripened cheeses.

It is obvious that in cheeses which are mottled with molds the molds play an important part in the ripening. The mold which ripens Roquefort cheese, and also Gorgonzola and Stilton cheeses, has been named "*Penicillium roqueforti* (Thom)" (*6*). Currie (*1*) has shown that it hydrolyzes fat and the resulting acids have the peppery or burning effect on the tongue and palate which is characteristic of Roquefort cheese.

In the making of Roquefort cheese, as in the making of Cheddar cheese, a rapid development of lactic-acid bacteria is necessary to bring about the proper physical condition of the curd in the various stages of manufacture. This acidity results from the growth of *Streptococcus lacticus*. The importance of the *S. lacticus* and *Penicillium roqueforti* in the making and ripening of Roquefort cheese was recognized by Thom, who

[1] In earlier publications on the flora of Cheddar cheese these organisms were called "*Bacterium casei*," the name which Freudenreich applied to the lactic-acid-producing rod forms of Emmental cheese. He failed to recognize the organisms as similar to the one which he had isolated from kefir, and which he called "*Bacterium caucasicum*"; later it received the name "*bulgaricum*." There does not appear to be sufficient cultural, morphological, and chemical differences between the lactic-acid-producing rod forms from cheese and from other sources to justify the use of two species names, although the strains isolated from cheese are more hardy than those isolated from oriental milk drinks in respect to growth at low temperatures and in respect to growth on ordinary media. In this paper the species will be designated as "*bulgaricum*," the term accepted by common usuage.

[2] Reference is made by number (italic) to "Literature cited," p. 233.

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made a study of mold-ripened cheeses. His observations (*6*) led him to believe that those two microorganisms were—

> capable of ripening Roquefort cheese without the introduction of other enzyme producing or flavor producing organisms.

In the Dairy Division experimental work, when cheese of the Roquefort type was obtained, the question was raised as to whether the bacterial flora had anything to do with the difficulties which arose; whether cheese made from sheep's milk, according to the Roquefort way in France, differed significantly in its bacterial flora from cheese made in a similar manner from cows' milk in America. Accordingly, a study of the bacterial flora of Roquefort cheese, imported and experimental, was undertaken.

METHODS OF CHEESE EXAMINATION

The method of examination was similar to that used in the bacteriological analyses of Cheddar cheese (*4*). Plate cultures were made on infusion agar, and for comparison numerous colonies were "fished off" into litmus skim milk. Representative cultures of every type of organism appearing in considerable numbers were saved for detailed study.

Cultures for study were also obtained by the following method: Milk cultures were inoculated from dilutions of a cheese emulsion, the dilution increasing from tube to tube by a ratio of 10. The dilutions ranged from 1 to 10 to 1 to 1,000,000,000. The lower dilutions were inoculated into tubes which had been modified as follows to separate the mold growth from the bacterial growth: Ordinary test tubes were drawn out to form a constriction beginning about 4 cm. from the bottom of the tube and filled with skim milk to about 2 cm. above the top of the constriction. Since oxygen is required for mold growth, the part of the culture below the constriction was always free from molds. By breaking the tube at the constriction it was possible to obtain a subculture of bacteria free from molds. As soon as a milk culture in any dilution showed evidence of bacterial growth, it was plated out to obtain a pure culture of the predominating organism. Many of the cultures thus obtained duplicated the cultures obtained by the plate method, but sometimes an organism was obtained which would be missed if plate cultures alone were made.

The pure cultures were studied morphologically and biochemically. The detailed study of the different groups of organisms will be presented in separate papers, for the bacteria of Roquefort cheese are not of types peculiar to that kind of cheese, but are ripening agents common to many other kinds, each species differing in importance in different kinds of cheese, according to the various conditions to which it is subjected.

BACTERIAL FLORA OF IMPORTED ROQUEFORT CHEESE

Bacteriological analyses of a number of imported Roquefort cheese have been made and the data are presented in Table I. Most of the imported cheese obtained for study was well ripened when the analysis was made. Cheeses 13, 146, 148, and 150 were not well ripened, but they must have been several weeks old, for *Streptococcus lacticus*, which certainly must have been present in the cheesemaking, had disappeared entirely from every sample examined.

TABLE I.—*The bacterial flora of imported Roquefort cheese*

Cheese No.	Number of organisms per gram of cheese.		Cheese No.	Number of organisms per gram of cheese.	
	Cheese streptococci.	*Bacterium bulgaricum.*		Cheese streptococci.	*Bacterium bulgaricum.*
13	31,000,000	100,000	137	600,000	1,000,000
15	600,000	400,000	139	10,000	1,000
17	13,000,000	4,000,000	141	100,000,000	1,000,000
18	30,000,000	33,000,000	143	1,000,000	1,000,000
131	1,400,000	145,000	146	210,000	250,000
133	17,000,000	.....	148	1,000,000	24,000,000
135	140,000,000	4,000,000	150	1,000	10,000

Two groups of bacteria were found to be the common flora of this type of ripened cheese: *Bact. bulgaricum*, and a group of organisms which here will be called "cheese streptococci." [1]

Table I shows that cheese streptococci in varying numbers, with 140,000,000 per gram as the highest number, were isolated from every one of the imported cheeses, and *Bact. bulgaricum* was isolated from all but one of them, with 33,000,000 per gram as the highest number. It most probably was present in the one cheese also, but failed to be isolated. Yeasts were isolated from a few of the cheeses, but in too small numbers to be considered of any significance.

BACTERIAL FLORA OF EXPERIMENTAL CHEESE MADE ACCORDING TO THE ROQUEFORT METHOD

For several years experimental cheese has been made in the Dairy Division according to the method by which Roquefort cheese is made, but with cows' milk instead of sheep's milk. The ripened cheese is very similar to the imported variety in appearance and flavor. The data obtained from a detailed study of the bacterial flora of the experimental cheese are given in Table II.

[1] The cheese streptococci are the subject of an accompanying paper (3). Culturally they are distinguished from *Streptococcus lacticus* by a failure to curdle litmus milk with the reduction of the litmus characteristic for that organism.

TABLE II.—*Bacterial flora of cheese made in the dairy division according to the Roquefort method. Number of organisms per gram of cheese*

Cheese No.	Age.	*Streptococcus lacticus.*	Cheese streptococci.	*Bacterium bulgaricum.*	Yeast.
1732...	These four samples were taken at progressive stages of the cheesemaking.	10,000,000	.....	.....	.....
1732...		20,000,000	.....	10	.....
1732...		6,500,000	.....	.....	.....
1732...		9,000,000	.....	.....	.....
1732...	1 day.....	100,000,000	.....	.....	.....
1732...	2 days.....	10,000,000	100,000,000	.....	.....
96. 115.	3 days.....	10,000	5,000,000	.....	.....
1732...	4 days.....	1,000,000	100,000,000	.....	.....
1732...	6 days.....	1,000,000	.....	.....	.....
1732...	8 days.....	100,000	100,000,000	.....	.....
1732...	12 days.....	100,000	31,000,000	.....	300
1732...	16 days.....	.....	.....	10,000	1,000
1732...	23 days.....	100,000	10,000,000	.....	.....
1789...	25 days.....	.....	7,500,000	14,000,000	100,000
1754...	35 days.....	.....	1,000,000	.....	1,000,000
1727...	38 days.....	.....	120,000	.....	.....
96. 121.	44 days.....	.....	1,000,000	.....	400,000
96. 17..	48 days.....	.....	40,000	100	100,000
96. 124.	2½ months.....	.....	10,000	1,000,000	10,000,000
96. 125.	3 months.....	.....	100,000	.....	2,500,000
1621...	3⅓ months.....	.....	.....	100,000	40,000
1601...	3¾ months.....	.....	.....	.....	1,000
1590...	4 months.....	.....	1,000,000	470,000	.....
96. 12..	4½ months.....	.....	2,150,000	1,000	.....
96. 113.	6 months.....	.....	10,000,000	1,000	.....
96. 129.	6⅓ months.....	.....	170,000	.....	.....

During the manufacturing of the cheese the *Streptococcus lacticus* which had been added as a starter was the only organism present in sufficient numbers to appear on the plates. These organisms gradually disappeared, and on the twenty-fifth day their numbers had so diminished that they no longer appeared on the plates.

A normal Roquefort cheese contains 4 per cent of sodium chlorid (NaCl) in 40 per cent of water, which can be regarded as a 10 per cent brine (*8*). An experiment was carried out to show the effect of a high concentration of salt on *Streptococcus lacticus*. Flasks of milk were inoculated with *S. lacticus*, incubated at 30° C. for about five hours, when sodium chlorid was added to make a concentration of 10 per cent. In five days all the organisms had been injured so that inoculations into tubes of litmus milk gave no growth. Later, a hardier strain of *S. lacticus* treated in the same manner was killed by the 10 per cent salt solution between the tenth and the seventeenth days. This experiment explains the complete disappearance of *S. lacticus* from Roquefort cheese during the first few weeks of ripening.

In the ripening cheese the cheese streptococci had multiplied to 100,000,000 per gram on the second day. A 6-months-old cheese still contained 10,000,000 of them, which demonstrates their hardiness in respect to a high concentration of the salt, quite unlike the sensitiveness of *S. lacticus*.

In the sample tested it was found that there were 10 cells of *Bact. bulgaricum* per gram of the curd just before cutting. By the twenty-fifth day the organism had multiplied to 14,000,000 per gram. Multiplication must have continued after the cheese was salted, on the sixth and eighth days. Later, this organism appeared in varying numbers. *Bacterium bulgaricum* is thus shown to be much more hardy than *Streptococcus lacticus* in respect to a high concentration of salt.

Yeasts were found more consistently in the cheese made in the Dairy Division than in the imported cheese, one cheese containing 10,000,000 per gram. The yeasts were either species of Saccharomyces which gave a gassy fermentation in dextrose, saccharose, and lactose, but not in maltose, or they were species which fermented dextrose alone.

In a general way it can be said that the bacterial flora of the cheese from the two sources was surprisingly alike. Fifty-three cultures of cheese streptococci isolated from domestic Roquefort cheese were subjected to many biochemical tests, and if slight differences in regard to fermentable substances were considered, they could be divided into seven strains. Forty-eight cultures isolated from the imported cheese were distributed among the same seven strains, with one additional strain. The case was the same with the *Bacterium bulgaricum*. They could be divided into many strains on the basis of their fermentation reactions, but no strain was peculiar to one or the other source.

When compared with the bacterial flora of Cheddar cheese, the kinds of bacteria are the same, but the number of bacteria in Roquefort cheese is decidedly lower. Roughly it may be said that there are about one-fifth as many in Roquefort as there are in Cheddar cheese. The flora is also less constant in Roquefort cheese. Tables I and II show that occasionally from both the imported and the experimental cheese a sample which had only a few thousand bacteria per gram was found.

Unlike Cheddar cheese, the number of bacteria in Roquefort cheese appeared to have no influence upon the ripening. For example, in Table II cheese 1601 had so few bacteria that there was no growth in the cultures inoculated with the 1 to 1,000 dilution. Nevertheless it was a good cheese, with a fairly well-developed flavor. This suggests that the bacteria, with the exception of *Streptococcus lacticus*, play a very insignificant part in the ripening of Roquefort cheese, an idea that is supported by the fact that if any cheese, or any part of a cheese, fails to develop the mold, ripening takes place exceedingly slowly, but the curd remains hard and retains the acid flavor resulting from the decomposition of the lactose by *S. lacticus*, and the Roquefort flavor does not develop.

The only condition in the Roquefort-cheese curd detrimental to the activity of bacteria is the high concentration of the salt. The comparatively low numbers of bacteria are undoubtedly due to the salt, and quite probably the activity of those which are able to exist in the cheese is restrained by the salt. So far as the development of flavor is con-

cerned, it appears to be a matter of indifference whether the bacteria are present or not, because the flavor produced by *Penicillium roqueforti* is so strong that it would mask any delicate flavors produced by the bacteria. Probably the flavor substances produced by bacteria, the acids, alcohols, and esters, are consumed by the mold. This study therefore confirms Thom's (*6*) opinion that the only bacteria essential to the making and ripening of Roquefort cheese is the *Streptococcus lacticus*.

INFLUENCE OF SLIME ON THE RIPENING PROCESS.

There is another problem in connection with the biology of Roquefort cheese ripening—viz, the influence which the organisms in the slime may have on the ripening process. Thom and Matheson (7) paraffined a large number of experimental Roquefort cheeses before slime had opportunity to develop, and they concluded that slime is not an essential factor in flavor production, but that it serves as an index of hygrometric conditions.

In the course of this study of the biology of Roquefort cheese ripening no evidence of any ripening changes proceeding from the exterior has been observed. When the cheese is cut through at any stage of the ripening, the appearance of the cut surface is uniform and no flavors have been observed in the outside layers more pronounced than in the interior. There is always a collection of moisture on the inside of the tin foil with which Roquefort cheese is covered—explained by the fact that the interior of the cheese has a higher temperature, owing to the fermentative processes, than the outside temperature of 7° to 10° C. at which the cheese is ripened. The condensation of moisture at the surface would be followed by a return movement toward the center to maintain the moisture equilibrium. This circulatory movement would tend to distribute through the cheese the enzyms which might be liberated by the organisms growing on the surface. This, it is argued by those who believe that the slime organisms are essential to Roquefort-cheese ripening, would bring about a uniform ripening throughout the cheese mass.

As a matter of general interest, the organisms making up the slime were studied. Immediately after the manufacture of the cheese *Oidium lactis* began to grow on the surface, and by the time the cheese was 2 days old it was well covered with oidium. On the sixth day the cheese was salted and the oidium was destroyed by the salt. Then came a growth of *Penicillium roqueforti* on the surface, which gave way to the growth of the typical reddish slime.

Smears were made of the slime from the surface of imported cheese and from the surface of experimental cheese in various stages of ripening. Microscopic examination showed that the slime was made up chiefly of bacteria, with scattered cells of yeasts, but in the smears from some of the cheeses yeast cells appeared in masses. Fragments of mycelium were occasionally seen. The bacteria in every smear were a mixture of rod

and coccus forms. The organisms that made up the slime were isolated by plating on various kinds of agar, plain agar, Czapek's agar, cheese-infusion agar, and plain agar to which lactic acid was added to eliminate the growth of bacteria and to favor the growth of yeasts and molds. The isolated bacteria were submitted to various tests commonly used for the differentiation of bacteria. The most frequently isolated organisms found in the slime from the imported and the experimental cheeses was a micrococcus which gave an abundant yellowish growth on agar slope, liquefied gelatin very slightly, decomposed urea, and stopped the fermentation of carbohydrates at a hydrogen-ion concentration of about $P_H = 5.6$. Many other cocci differing only slightly from the description above were isolated from the slime. Micrococci identical with these cocci have been isolated commonly from aseptically drawn milk (*2*). It appears, then, that the udder is the source of the predominating flora of the cheese slime. There is apparently a selection of certain strains of udder cocci. The majority of them curdle milk, but no micrococcus isolated from cheese slime produced more than a slight acidity.

Another coccus fairly common in the slime from both imported and experimental cheese was exceedingly difficult to maintain on agar slopes. It formed a faint growth on agar and failed to attack the nitrogenous and carbohydrate test substances in broth cultures. It was surprising to find an organism that was capable of withstanding the rigorous conditions in the cheese slime and yet so delicate that it could scarcely be maintained under artificial cultivation.

The most frequently isolated rod form liquefied gelatin, decomposed asparagin, and gave an alkaline reaction in broths containing various carbohydrate test substances. Other rod forms differed slightly from this one.

Besides the types which have been briefly described as typical slime organisms, various kinds of cocci and rods were isolated only once or twice, which do not seem to be characteristic of the slime. For example, *Bacterium bulgaricum* was isolated from the slime once. Occasional colonies of mold, most frequently *Penicillium roqueforti*, appeared in the cultures.

None of the cheese-slime organisms were able to bring about any pronounced changes in milk in pure culture, but when pure culture of several types were inoculated together into milk their associative action digested the casein very slowly.

It would be a difficult matter to prove that these sluggish proteolytic enzyms either do or do not influence the ripening of the cheese. Gratz and Szanyi (*5*) made a careful chemical investigation of the action of the enzyms of the slime upon the ripening of the interior of hard cheeses of the Ovar and Trappist varieties on which the slime often becomes heavy. The authors concluded that in hard cheese of the type studied no ripening proceeds from the outside. The relative inactivity of the

organisms of the slime, as compared with the vigorous activity of the *Penicillium roqueforti* suggest that in Roquefort cheese also the slime organisms are of minor importance, if indeed they have any influence in the cheese ripening.

It is said that if the *Penicillium roqueforti* fails to develop, there is, nevertheless, a softening of the curd after a long period of ripening. In such case the ripening is undoubtedly brought about by the bacteria of the interior of the cheese, probably aided to a considerable extent by the enzyms from the slime; but a ripe cheese of that kind is not a typical Roquefort cheese.

The Roquefort-cheese slime is normally of a reddish color, but no organisms producing red pigment were isolated from it, although apparently all forms seen in the smears grew in the plates. The only explanation that can be offered is that the cocci, rod forms, and yeast cells, all containing more or less yellow-and-orange pigment, may produce a reddish tinge when mixed in mass. It is quite possible that the pigment production of one or all of those species is altered by the intimate association with the other species.

SUMMARY

The microorganisms essential for the manufacture and ripening of Roquefort cheese are *Streptococcus lacticus* and *Penicillium roqueforti*.

Streptococcus lacticus decomposes the lactose during the manufacture of the cheese and thus produces the lactic acid necessary for the cheese making. These organisms disappear from the cheese after about two or three weeks, being killed by the high concentration of sodium chlorid.

The remaining flora of Roquefort cheese consists of cheese streptococci and *Bacterium bulgaricum*, organisms which are found in all kinds of ripening cheese. These organisms do not have any significant part to play in the ripening of Roquefort cheese.

The cheese slime consists of characteristic types of micrococci, rod forms, and yeast cells. The enzyms from the slime do not appear to be essential to the ripening of the cheese.

The flora of both the interior and the slime of the experimental cheese was identical with the flora of the interior and the slime of the imported cheese.

If the maker of Roquefort cheese will inoculate properly with *Streptococcus lacticus* and *Penicillium roqueforti*, and provide the proper condition of manufacture and ripening, he need have no other concern about biological ripening agents.

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A STUDY OF THE STREPTOCOCCI CONCERNED IN CHEESE RIPENING

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INTRODUCTION

In a general way streptococci may be divided into three groups: First, the pathogenic streptococci, which differ among themselves in virulence and in their predilection for certain organs, as well as in certain biochemical reactions. Second, those streptococci which are common in the udder, in the saliva, and in the intestines. These, so far as we know, differ from the first group chiefly in their lack of virulence. Third, the milk-souring streptococci (*Streptococcus lacticus*).

It is the purpose of this paper to present streptococci in another rôle—viz, as the producers of flavor substances and other ripening changes in food prepared for consumption by fermentation, and particularly in the ripening of cheese. In this connection the streptococci are important as one of the factors concerned in rendering palatable many articles of food in use throughout the world. But they are unobtrusive, and demand little attention—only that the proper conditions for their growth shall be provided. The manufacturer of the food meets this demand without knowing why; he only knows that if he follows such and such a procedure he will obtain the desired results. Therefore the flavor-producing streptococci have escaped notice.

Streptococci from all four of the sources mentioned differ so slightly that there has been much discussion as to whether they are varieties of the same species. Several species, however, all belonging to the genus Streptococcus, have come to be generally recognized. The accepted definition of the genus as given by the Winslows (*23, p. 141*)[2] is as follows:

> Parasites. Cells normally in short or long chains (under unfavorable cultural conditions, sometimes in pairs and small groups, never in large packets). Generally stain by Gram. On agar streak, effused translucent growth, often with isolated colonies. In stab culture, little surface growth. Sugars fermented with formation of large amount of acid. Generally fail to liquefy gelatin or reduce nitrates.

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[1] The writer is indebted to her collaborators in the Dairy Division for hearty cooperation whenever the investigation was found to extend into the special field of research of any one of them. The gas analyses were made by Dr. W. M. Clark; Dr. J. N. Currie made the volatile-acid determinations; Mr. H. C. Jordan made the experimental cheese of the Cheddar type; and Mr. K. J. Matheson and Mr. F. R. Cammack made the soft-cream cheese.

[2] Reference is made by number (italic) to "Literature cited," p. 251–252.

The streptococci concerned in cheese ripening, *S. lacticus* and the other varieties described in this paper, comply with the generic description in every detail except in regard to parasitism. The cultural characteristics of the group are too well known to be described further in this paper, which has to do with a classification of those streptococci that act as ripening agents in cheese and other food substances.

Streptococci agreeing with the genus description given above, and differentiated from *S. lacticus* by their growth in milk culture, have been isolated from many kinds of cheese by several investigators. As the data to be presented will show, the manner of growth in litmus milk is a reliable characteristic for the differentiation of *S. lacticus* from other distinct varieties of streptococci which are normal inhabitants of various kinds of cheese. Therefore it may be safely assumed that the investigators in question were dealing with varieties of streptococci other than *S. lacticus*. For convenience in discussion in this paper, all other types of streptococci as distinguished from *S. lacticus* will be called "cheese streptococci." Henrici (*11*) found them in Schweitzer, Edam, Gouda, Port du Salut, American, Lauterbacher, Monsheimer, Münster, Limburg, Spunden, Schloss, Cantal, Brie, cream, and Neufchâtel cheeses. Troili-Petersson (*21*) found them in what the Germans call Swedish Güterkäse. Eldredge and Rogers (*4*) found streptococci in Emmental cheese. Evans, Hastings, and Hart (*8*) reported that they are active agents in the ripening of Cheddar cheese. In an accompanying paper (*7*) they are shown to be one of the predominating types of bacteria in Roquefort cheese.

Thus, it has been shown that cheese streptococci are present in both hard and soft cheeses made by various methods in different countries. Freudenreich (*9*) found streptococci that were identical with one of the varieties described in this paper to be active in the preparation of "*kefir*," and Saito (*17*) found that these same organisms were active in the fermentation of the mash from which the Japanese condiment "*soya*" is prepared. The writer has found similar streptococci in the Chinese "*toku*," or soybean cheese. It is probable that they are active in the fermentation of other foods.

The source of the cheese streptococci is suggested by the fact that other bacteria concerned in cheese making and cheese ripening, *Bacterium bulgaricum* and *S. lacticus*, are normal inhabitants of the intestines and saliva of animals. Some strains of the cheese streptococci agree in every detail with the published descriptions of *S. mitis;* others agree with the descriptions of *S. faecalis*, both of which are streptococci normally inhabiting the mouth and intestines of men and domestic animals. Other cheese streptococci differ from the species mentioned only in fermenting slightly different combinations of test substances; hence, it appears that the cheese streptococci get into the milk with the filth of the stable. Those which multiply in cheese must be particularly hardy

strains, for they develop in the presence of the by-products of *S. lacticus* and other cheese organisms; in certain types of cheese they develop in the presence of a high concentration of sodium chlorid (10 per cent in Roquefort cheese); in the manufacture of other types of cheese they survive the "cooking" of the curd (from 52° to 60° C. for about an hour in the making of Emmental cheese).

The tests ordinarily used for the differentiation of streptococci reveal nothing peculiar about the cheese streptococci. After applying the tests, therefore, it was necessary to study the cultures biochemically along such lines as observation of their conduct pointed out to be possibly fruitful. The result has been the recognition of two distinct species of cheese streptococci in addition to *S. lacticus*. Having recognized the species, and knowing something of their food requirements, the way is opened to a chemical investigation of their by-products which should yield interesting results. This paper presents the bacteriological differentiation between *S. lacticus* and the two other species, with a few fundamental facts about their peculiar physiological activities and the relation of the organisms to cheese ripening.

METHODS OF STUDY

All cultures were incubated at 30° C. For the study of morphology smears were made from 24-hour cultures from the condensation water of agar slopes. The smears were stained according to the Gram-Weigert method, and drawings were made with the aid of the camera lucida.

The methods for determining the liquefaction of gelatin, decomposition of asparagin and urea, and reduction of nitrate are described in a previous publication (*6*) to which the reader is referred. All cultures studied reacted negatively to these tests, and therefore they will not be mentioned again. The fermentation reactions were determined in the following test substances: Dextrose, lactose, saccharose, raffinose, salicin, mannite, inulin, and glycerin. Every strain reacted positively to dextrose and negatively to inulin. These substances therefore had no differential value and will not be mentioned again. One per cent of the test substance was added to a yeast-peptone broth made of 10 gm. of peptone, 5 gm. of desiccated yeast, and 5 gm. of dibasic potassium acid phosphate in a liter of water. Desiccated yeast was used in place of the usual meat extract because it is in general use in these laboratories and not because it was peculiarly adapted to the problem at hand. Sodium hydroxid was added to bring the medium to a hydrogen-ion concentration of $P_H = 6.8$. The cultures were incubated for seven days.

The hydrogen-ion concentration was then determined by the colorimetric method, with the standard solutions described by Clark and Lubs (*1*) and the indicators described by them (*14*).

The same yeast broth, without addition of a fermentable substance, and without the addition of sodium hydroxid, was found to be a very

useful medium for the study of the streptococci. The hydrogen-ion concentration of such medium is about $P_H = 6.0$.

Cultural characteristics were determined in litmus skim milk, and in litmus skim milk to which 0.5 per cent of peptone had been added.

Another very useful medium was trypsin-digested milk. One per cent of commercial trypsin was added to the milk, and it was kept at about 40° C. for two or three hours. Sodium hydroxid was added to bring the reaction to about $P_H = 7.2$ at the beginning of the digestion and several times afterward to neutralize the liberated amino acids. The final hydrogen-ion concentration of the medium was about $P_H = 6.2$. The medium was filtered, heated in the autoclave as for sterilization, refiltered, put into tubes or flasks and sterilized for use.

Volatile-acid production was determined in milk cultures. Most of the cultures were grown in 500 c. c. of skim milk, but a few of the first determinations were made in cultures grown in 200 c. c. of skim milk. For these the data have been calculated for 500 c. c. Duplicate cultures were analyzed after a varying length of incubation, and the results showed that after the first week there is little or no increase in volatile acids. The subsequent determinations were made after from 7 to 15 days' incubation. Dilute phosphoric acid was added to the culture until a blue color was given with Congo red, to release any volatile acid that might be in combination. In some preliminary determinations sulphuric acid had been employed for the purpose, but it was decided that small quantities of formic acid resulted during the distillation from the action of sulphuric acid on some constituent of the milk, possibly casein. The cultures were distilled with steam until 2,000 c. c. of distillate were collected. At first an effort was made to distill under reduced pressure to hasten the process, but it had to be abandoned because the cultures foamed so freely.

The distillate was neutralized with barium hydroxid [$Ba(OH)_2$], evaporated to a small volume, decomposed with sulphuric acid (H_2SO_4) and distilled by the method of Duclaux (*3*). This method serves both to identify and to estimate the volatile acids present. Although some of the data indicated traces of acids of lower molecular weight than acetic, probably formic, and also traces of acids higher than acetic, possibly butyric, which might arise from a slight hydrolysis of the fat in the milk, it seems justifiable to conclude that acetic acid is the only volatile acid produced by either *S. lacticus* or the cheese streptococci in their normal processes of metabolism in milk culture.

STREPTOCOCCUS LACTICUS

This organism has been known in the literature under the names "*Bacterium güntheri*," "*Bacterium lactis acidi*," and various other names, but is now generally designated as "*Streptococcus lacticus* (Kruse)." Its close relationship to the other varieties of streptococci argues forcibly

against classifying it with the rod forms, as the generic name "Bacterium" implies. Undoubtedly some authors have included under the name "*Streptococcus lacticus*" the varieties of streptococci which the results of this study show to be quite distinct.

S. lacticus has a tendency to form elongated cells with pointed ends, and to separate in pairs rather than remain together in long chains. These characters are variable, however, according to the conditions under which the organism is grown. The other cheese streptococci also form elongated cells under certain conditions, and some strains of them are no more inclined to form chains than is *S. lacticus;* hence, morphology will not distinguish *S. lacticus*.

A summarized description of the *S. lacticus* group is given in Table I. The cultures included in the table were isolated from various sources. Culture 96mz was isolated from a commercial starter. Culture 96ga was from an experimental Roquefort cheese, and is probably the same strain as culture 96mz, the starter culture. Culture 2ak was from unpasteurized milk. Cultures 96hr and 96hs were from cream from two dairies. Cultures 2am, 2al, 2ao, 2an, and 2ap were from pasteurized milk from five dairies. Culture 2ab was from Cheddar cheese. Culture 96ht was from soybean cheese imported from China.

The growth of *S. lacticus* in litmus milk is characteristic. This streptococcus begins to reduce the litmus before the reddening appears, and before the milk coagulates, the reduction is complete beneath the pink surface layer. Esten (*5*) noted this reaction. Sherman and Albus (*19*) have recently confirmed it as a typical reaction for *S. lacticus*, although other types of bacteria also give the same reaction. The data to be presented in this paper show that the characteristic reduction of litmus, which is correlated with other physiological activities, serves well in routine work for a differentiation of it from other varieties of cheese streptococci. In an active culture of *S. lacticus* curdling takes place in less than 24 hours. Weakened strains require a longer time, or they may fail to curdle the milk.

Another characteristic of *S. lacticus* in milk cultures is the formation of crystals.[1] White specks or crystals are familiar in well-ripened Cheddar cheese, and occur also in well-ripened Roquefort cheese. Dox (*2*) identified the crystals from Roquefort cheese as tyrosin. Crystals isolated from Cheddar cheese and from pure-milk cultures of streptococci have been identified as calcium compound of an amino acid. Crystals were formed by all but 1 of the 12 strains of *S. lacticus* which served for this study.

In one of the earliest descriptions of *S. lacticus*, or "*Bacterium lactis acidi*," as he called it, Leichmann (*13*) reported that it produced lactic acid with no volatile acid. Jensen (*12*) and others have since shown

[1] For the determination of crystal formation milk cultures are covered tightly with tin foil over the cotton plug and incubated for six weeks or longer.

that small quantities of volatile acids, chiefly acetic, were produced by this organism. In an earlier work (*10*) it was shown that some strains of streptococci which did not give a reduction of litmus characteristic of *S. lacticus* produced large quantities of acetic acid in milk cultures. This suggested that differences in acetic-acid production might serve as a basis for the differentiation of cheese streptococci. Therefore volatile-acid determinations were made on milk cultures of five strains of *S. lacticus*, four of which produced between 9.90 and 11.27 c. c. *N/10* acetic acid in 500 c. c. of milk. The other strain produced 7.25 c. c. of acetic acid. Cultures of *S. lacticus* agree, therefore, in producing a small and fairly constant quantity of acetic acid in milk cultures, equivalent to about 0.12 gm., calculated for 1 liter of milk.

Jensen (*12*) found that *S. lacticus* could develop in peptone broth without any carbohydrate, and that when growing thus it was able to decompose the peptone with the formation of amino acids and ammonia. This is a definite reaction when measured in terms of the hydrogen-ion concentration. Cultures of *S. lacticus* inoculated into carbohydrate-free yeast peptone broth with a hydrogen-ion concentration of $P_H = 6.0$ grew abundantly, reducing the hydrogen-ion concentration to $P_H = 6.8$. This indicates a vigorous proteolytic activity. Two cultures varied slightly from this final hydrogen-ion concentration; and two cultures, 96ga and 2am were atypical in failing to grow in the carbohydrate-free medium.

S. lacticus cultures isolated from Cheddar cheese were reported in an earlier work (*8*) to be positive in their fermentation of lactose in broth cultures, negative in glycerin broth, and either positive or negative in salicin, mannite, and sucrose broth. The fermentation reactions of the *S. lacticus* cultures isolated for the present study agreed with the earlier description. The order of availability of the test substances was the same as noted in the earlier publication—viz, lactose, salicin, mannite, sucrose. Glycerin and raffinose were not fermented. The fermentation reactions of each of the 12 cultures which were studied in detail ware presented in Table I. The final hydrogen-ion concentrations are given only for those cultures which hydrolyzed the test substance. The minus sign (−) does not indicate a failure to grow, but a failure to form acid. As would be expected, if the test substance is not attacked, the same reaction would take place as in the plain-yeast-peptone broth. The table shows that there is a considerable variation in the final hydrogen-ion concentration of any one strain in the different broths, and also that there is a variation in the final hydrogen-ion concentration of the different strains in any one of the broths. The final hydrogen-ion concentrations varied from $P_H = 4.0$ to $P_H = 5.0$. The same was the case in dextrose broth, not included in the table. The succeeding tables show that the same thing is true of the other cheese streptococci, and that the different species cover the same range of variation. Therefore the determination of the final

hydrogen-ion concentration in the broth medium used in this study does not aid in the classification of the streptococci under consideration. It may be possible to devise a medium in which the final hydrogen-ion concentration of the different varieties would be characteristic.

TABLE I.—*Characteristics of Streptococcus lacticus*

Culture No.	Changes in litmus milk.		Crystals in milk.	Quantity of $N/10$ acetic acid in 500 c. c. of milk.	Final P_H values in peptone-yeast broth (P_H=6.0).	Final P_H values in fermented broths.[a]					
	Curdling.	Reduction.				Lactose.	Salicin.	Mannite.	Sucrose.	Raffinose.	Glycerin.
	Days.			*C. c.*							
96mz ..	2	Complete .	—	7.25	.....	5.0	—	—	—	.....	—
96ga ...	2	...do.....	+	9.90	No growth.	4.1	—	—	—	—	—
2am ...	1	...do.....	+	.....	...do...	4.7	—	—	—	—	—
2ak....	1	...do.....	+	.....	6.8	4.6	4.7	—	—	—	—
96hr ...	1	...do.....	+	11.27	6.8	5.0	5.2	—	—	—	—
96hs ...	1	...do.....	+	10.65	.....	5.0	5.2	—	—	—	—
2al.....	2	...do.....	+	.....	6.8	4.7	4.7	4.9	—	—	—
2ao	1	...do.....	+	.....	6.8	4.5	4.7	4.8	—	—	—
2an....	1	...do.....	+	.....	6.6	4.6	4.6	—	4.2	—	—
2ab....	1	...do.....	+	10.56	6.8	4.6	4.7	4.6	4.1	—	—
96ht ...	2	...do.....	+	.....	6.9	4.6	4.6	4.6	4.4	—	—
2ap....	1	...do.....	+	.....	6.8	4.6	4.6	4.8	4.4	—	—

[a] The minus sign (—) indicates failure to form acid.

STREPTOCOCCUS X

It was stated above that after applying to all the streptococcus cultures isolated from cheese the biochemical tests which served to characterize *S. lacticus*, two other species were recognized. One of them is designated *Streptococcus X*, which is merely a tentative appellation to serve until a better knowledge of the milk and cheese streptococci is at hand. The relation of this group of organisms to cheese ripening will be discussed later in this paper. To anticipate that discussion it may be stated here that *Streptococcus X* brings about changes in ripening cheese which are easily distinguishable from the changes caused by *S. lacticus*. This is one of the most forceful arguments to justify considering it a species distinct from *S. lacticus*.

Morphologically *Streptococcus X* differs slightly from *S. lacticus*. In the condensation water from agar slopes the cells occur commonly in pairs and under some conditions the cells are elongated and slightly pointed at the ends. But *Streptococcus X* has more of a tendency than *S. lacticus* to form groups of from two to seven cells. This grouping of cells often suggests that they may belong to the micrococci rather than to the streptococci, but short chains of from four to eight cells are to be found in almost every culture. In dextrose broth long chains are formed, which classifies the group definitely with the streptococci.

Streptococcus X was found to be abundant in Cheddar and Roquefort cheese. Thirteen representative strains were selected for detailed study. Four of them were from Cheddar, and nine were from imported and experimental Roquefort cheese. In Table II, where the data for this group are summarized, the strains numbered 2 were from Cheddar cheese, and those numbered 96 were from Roquefort cheese.

TABLE II.—*Characteristics of Streptococcus X*

Culture No.	Changes in litmus milk.		Crystals in milk.	Quantity of *N/10* acetic acid in 500 c. c. of milk.	Final P_H values in peptone yeast broth (P_H=6.0).	Final P_H values in fermented broths.[a]					
	Curdling.	Reduction.				Lactose.	Salicin.	Glycerin.	Mannite.	Sucrose.	Raffinose.
	Days.			*C. c.*							
2ac....	4	None....	—	49.39	6.8	4.6	4.7	—	5.2	—	—
2ae....	4	...do.....	—	67.75	6.8	4.8	4.0	—	—	4.0	—
2aa....	5	...do.....	—	70.00	6.8	4.5	4.2	6.1	—	—	—
96ab...	(*b*)	...do.....	—	.....	6.7	3.8	5.8	5.9	—	—	—
96et...	6	Partial...	—	63.76	6.8	4.7	4.8	6.1	—	—	—
96hb...	12	None....	—	62.34	6.8	4.7	4.7	6.5	—	—	—
2ai.....	5	Slight...	—	60.12	6.6	5.1	5.4	6.5	—	—	—
96dc...	6	Partial...	—	65.50	6.8	4.6	4.7	5.4	4.9	—	—
96hi....	3	Slight...	—	.....	6.8	4.0	4.5	5.6	4.8	—	—
96bu...	5	...do.....	—	58.25	6.8	4.0	4.1	5.6	0	3.8	—
96fh....	2	None....	—	.....	6.8	4.6	4.3	6.2	0	4.3	—
96dq...	6	Slight...	—	57.88	6.8	4.5	4.5	5.2	5.2	4.2	—
96gx...	3	...do.....	—	65.24	6.8	4.2	4.7	6.4	5.1	5.2	—

[a] The minus sign (—) indicates failure to form acids. [b] Not curdled in 14 days.

Streptococcus X requires a longer time than *S. lacticus* to curdle litmus milk. Table II shows that the cultures varied in that respect from 2 to 12 days. There is a partial reduction, or no reduction of the litmus. In peptone milk curdling usually takes place in one day. There is no crystal formation in milk cultures.

The most striking differentiation between *S. lacticus* and *Streptococcus X* is the large quantity of acetic acid formed in milk cultures by the latter. The quantity of acetic acid produced in milk cultures was determined for 10 strains of this group. With the exception of one culture, 2ac, which produced 49.39 c. c. *N/10* acetic acid in 500 c. c. of milk, which was somewhat less than was characteristic for the group, the quantity was fairly constant, varying in eight cultures between 57.88 and 67.75 c. c. Culture 2ac produced 70 c. c., slightly more than the characteristic quantity of acetic acid. If calculated for 1 liter of milk, the characteristic quantity of acetic acid produced by this group of streptococci is equivalent to 0.7 to 0.8 gm. per liter, or approximately six times the quantity of acetic acid produced by *S. lacticus*.

The reduction of the hydrogen-ion concentration in peptone-yeast broth with an initial concentration of $P_H = 6.0$ was precisely the same for *Streptococcus X* as for *S. lacticus*. Two of the thirteen cultures varied slightly from the characteristic final $P_H = 6.8$.

The order of availability of the fermentable test substances is somewhat different from that for *S. lacticus*. In both groups lactose is fermented by every strain. The fermentation tests were made for 27 strains of *Streptococcus X* in addition to the 13 strains included in the table. Every one of the 40 strains fermented salicin. All but 2 of the strains, 2ac and 2ae (Table II), or 95 per cent, fermented glycerin; 27, or 67.5 per cent, fermented mannite; and 14, or 35 per cent, fermented sucrose. The order of availability of the test substances of *Streptococcus X* is, therefore, lactose and salicin, glycerin, mannite, sucrose. The high percentage of positive reactions in glycerin broth characterizes this group as compared with *S. lacticus*. Raffinose was not fermented.

Under the conditions of the tests, the final hydrogen-ion concentrations in fermented broths varied widely, and failed to characterize the group. In glycerin broth the final hydrogen-ion concentration was notably less than in the other fermented broths.

STREPTOCOCCUS KEFIR

This organism was first isolated by Freudenreich (*9*), who called it "*Streptococcus b.*" Migula (*15*) gave it the name "*Streptococcus kefir.*" Under the name "*Bacterium soya,*" Saito (*17*) described an organism which is apparently identical with this one. Out of consideration for priority in names, this group of organisms should be called "*Streptococcus kefir.*"

There is nothing characteristic about the morphology of *S. kefir*. In the condensation water from agar slopes it occurs in pairs, and occasionally short chains are formed. It has not so pronounced a tendency to group formation as *Streptococcus X*, but occasionally groups of from four to six cells are to be found.

Freudenreich found *S. kefir* to be a predominating organism in kefir. Saito found it to be one of the organisms concerned in the fermentation of the mash in the preparation of the Japanese condiment *soya*. It has been found in very great numbers in Roquefort and Cheddar cheese. No doubt a further search for this organism will show that it is important in the fermentation of other kinds of food substances, as well as other kinds of cheese. In Table III, where the data for this group are summarized, the strains numbered 96 are from imported and experimental Roqufort cheese, and the strain numbered 2 is from Cheddar cheese.

In litmus milk growth is slow, and the milk is rarely brought to curdling. Usually the acidity is only slight. There is no reduction of litmus. In peptone milk the behavior of *S. kefir* is striking and distinguishes it from all other known streptococci of food and dairy products. The peptone milk is acidified rapidly, with curdling in from three to six days. The curd is rent with escaping gas, and gas bubbles can be seen rising in rapid succession.

TABLE III.—*Characteristics of Streptococcus kefir*

Culture No.	Changes in litmus milk.		Changes in peptone milk.		Behavior in peptone yeast broth (P_H= 6.0).	Final P_H values in fermented broths.[a]					
	Acidity.	Reduction.	Curdling.	Gas.		Lactose.	Sucrose.	Raffinose.	Salicin.	Mannite.	Glycerin.
			Days.								
96gq...	Slight.....	None.	3	+	No growth.	4.0	4.6	—	—	—	—
2aj.....	...do.....	...do..	4	+	...do...	5.0	5.2	—	—	—	—
96aw..	Not curdled.	...do..	3	+	...do...	4.8	4.1	6.4	—	—	—
96ew..	Slight.....	...do..	6	+	...do...	5.4	4.7	5.3	—	—	—
96fi....	Not curdled.	...do..	2	+	...do...	4.8	4.2	4.9	—	—	—
96dd...	Slight.....	...do..	3	+	...do...	4.0	4.3	6.0	4.5	—	—

[a] The minus sign (—) indicates failure to form acids.

The best medium found for the growth of *S. kefir* is digested milk, in which there is a vigorous gas production. Quantitative and qualitative gas analyses were made according to the method described by Rogers, Clark, and Davis (*16*). In 20 c. c. of the digested milk, with an initial hydrogen-ion concentration of $P_H = 6.6$, 26.80 c. c. of gas were produced by culture 96fi. This culture produced only 3.82 c. c. of gas in 20 c. c. of dextrose-yeast broth, and 2.82 c. c. of gas was produced in 20 c. c. of a lactose-yeast broth culture of this same strain. The growth in the sugar broths was abundant, with a vigorous acid formation. In all these media the gas produced was pure carbon dioxid, with only a trace of some other gas, undoubtedly due to experimental error. The source of the carbon dioxid has not yet been determined.

The gas production of this group of cheese streptococci served to identify it with the organisms described by Freudenreich (*9*) and Saito (*17*). Freudenreich reported that his streptococcus produced 12 c. c. of gas in 50 c. c. of a lactose-peptone broth; lactic acid also was formed. Saito reported that his organism produced carbon dioxid, lactic acid, and alcohols.

Culture 96gq of *S. kefir* grown in 500 c. c. of skim milk produced 57.14 c. c. of *N/10* acetic acid. The milk had not been acidified to the point of curdling. In 500 c. c. of a milk culture to which 0.5 per cent of peptone had been added culture 96gq produced 80.50 c. c. of *N/10* acetic acid, equivalent to 0.97 gm. of acetic acid per liter.

S. kefir will not grow in peptone-yeast broth without a carbohydrate. Lactose and sucrose were fermented by every one of the 23 strains on which the tests were made. Raffinose was fermented by most of the strains. Salicin was fermented by only one strain, culture 96dd. The order of availability of the test substances for *S. kefir*, therefore, is as follows: Lactose and sucrose, raffinose, salicin. Mannite and glycerin are not fermented. To judge from the limited number of cheese streptococci which have been studied, it appears that the fermentation of lactose and

sucrose with failure to ferment mannite and glycerin characterizes a cheese streptococcus as a gas producer, for no strain which has fermented this combination of test substances has failed to give a gas production in peptone milk. The fermentation of raffinose by most strains also distinguishes *S. kefir* from *S. lacticus* and *Streptococcus X*.

COMPARISON OF S. LACTICUS, STREPTOCOCCUS X, AND S. KEFIR

S. lacticus, *Streptococcus X*, and *S. kefir* are three well-defined species. *S. lacticus* is characterized by a vigorous reduction of the litmus in milk cultures, which precedes the curdling, and is complete beneath the pink surface layer; about 0.12 gm. of acetic acid is produced per liter of milk; crystals are formed in milk cultures by a large percentage of the strains; and there is a vigorous production of alkali by the majority of strains grown in peptone broth free of fermentable substance. *Streptococcus X* is characterized by a slower production of acidity in milk cultures, with only a partial reduction of the litmus; no crystals are formed in milk cultures; about 0.75 gm. of acetic acid is produced per liter of milk; and all strains produce alkali in peptone broth. *S. kefir* is characterized by a faint growth in milk cultures; no growth takes place in peptone broth; the acetic acid production is similar to that of *Streptococcus X;* and there is a vigorous production of gas in peptone milk or digested milk cultures.

A summarized description of the three species of streptococci is given in Table IV, where the characteristic reactions can be compared. Average values are given in those columns of the table where the test is expressed in numbers. Considering all the tests, the fermentation reactions gave the least aid in this classification. The order of availability, however, is distinctly different for each species, and there are certain individual reactions which are more or less characteristic. For example, fermentation of glycerin would indicate that a streptococcus belonged to the *Streptococcus X* group; fermentation of raffinose would indicate that a strain belonged to the *S. kefir* group; fermentation of sucrose is also somewhat characteristic of *S. kefir*, for it has been fermented by every strain of this group, whereas the majority of strains of the other two groups failed to ferment sucrose.

TABLE IV.—*Comparative description of S. lacticus, Streptococcus X, and S. kefir*

Organism.	Changes in litmus milk.		Crystals in milk.	Changes in peptone milk.		Quantity of N/10 acetic acid in 500 c. c. of milk.	Final P_H values in peptone yeast broth (P_H=6.0).	Fermentation of [a]—					
	Curdling.	Reduction.		Curdling.	Gas.			Lactose.	Salicin.	Mannite.	Sucrose.	Raffinose.	Glycerin.
				Days.		*C. c.*							
Streptococcus lacticus.	1 day.....	Complete	±	1	—	10.00	6.8	+	±	±	±	—	—
Streptococcus X...	5 days.....	Partial..	—	1	—	62.00	6.8	+	+	±	±	—	±
Streptococcus kefir	Slight acidity.	None....	—	3	+	[b] 80.00	No growth	+	±	—	+	±	—

[a] The minus sign (—) indicates failure to form acids. [b] Peptone was added to the milk.

OTHER STRAINS

Not all the cheese streptococci can be classified in one or another of the three groups which are described above, although most of the strains which were studied agreed in every detail with those types. The irregular strains will not be described in this paper for lack of sufficient data. The study of a larger number of strains of streptococci from a wider variety of fermented foods will most probably show that there are other distinct types of flavor-producing streptococci, to which some of these irregular strains may belong. Others are probably atypical strains.

A few strains isolated from Roquefort cheese were non-lactose-fermenting and agreed in every way with the descriptions of *S. equinus*, but they have not been encountered frequently enough to suggest that they may be of importance in cheese ripening.

STREPTOCOCCUS X AND S. KEFIR AS RIPENING AGENTS OF CHEDDAR CHEESE

As a result of an earlier work on Chedder cheese (*8*), the conclusion was drawn that this type of cheese could not ripen normally without the activity of several strains of streptococci. When the milk was pasteurized for cheese making, the streptococcic flora consisted entirely of *S. lacticus* used as a starter. Such a cheese never developed the delicate flavor typical of the Cheddar type, but instead it retained the acid flavor of the curd until the influence of *Bacterium bulgaricum* became evident in the late stages of ripening. The texture of the pasteurized-milk cheese was also unlike that of raw-milk cheese. The pasteurized-milk cheese remained white, opaque, and somewhat soggy, whereas the raw-milk cheese became almost translucent, and developed a yellowish color. The addition of one other strain of cheese streptococcus, together with *S. lacticus* in the starter, modified the pasteurized-milk cheese favorably; but the typical Cheddar flavor was never obtained in the earlier series of experiments. It was shown that several varieties of streptococci could be isolated from a ripening Cheddar cheese made from raw milk, and it appeared that the several varieties were necessary to produce typical flavors.

Two species of cheese streptococci have now been differentiated from *S. lacticus*. A second series of experimental pasteurized cheese has been made to show the effect of *Streptococcus X* and *S. kefir* on the ripening process, with definite results. The studies of only three of the cheeses need be mentioned to illustrate these results. The milk was pasteurized for all three cheeses. One cheese was inoculated with a starter of *S. lacticus*. It developed the acid flavor, and retained the white, opaque, somewhat soggy curd characteristic of a cheese made in this manner. The second cheese was made in just the same way, but had added to it a starter of *Streptococcus X* in addition to the *S. lacticus* starter. This cheese was decidedly milder in flavor, the mildness being apparent as a

lesser acidity. In color and texture it was noticeably more like a raw-milk cheese after about two months of ripening. Samples were submitted to several persons, who agreed that the experimentally inoculated cheese was preferable to the control. The third cheese was made in the same way, but had starters of *Streptococcus X* and *S. kefir* in addition to the *S. lacticus* starter. After two months' ripening it was unquestionably the best cheese of the three. In fact, a Cheddar cheese can rarely be obtained in market altogether as pleasing as this cheese because the undesirable organisms in milk when it comes to the factory commonly give to cheese "unclean" flavors. In the pasteurized-milk cheese all undesirable organisms have been destroyed, and in the case of the cheese in question, a part, at least, of the flora necessary for developing the delicate flavors typical of a Cheddar cheese made from raw milk had been restored. For a few weeks during the third and fourth months of ripening the flavor of the one inoculated with cheese streptococci did not compare so favorably with that inoculated with *S. lacticus* alone. After four months' ripening at about 23° C. the cheeses were placed in a refrigerator where the temperature varied from 12° to 15° C. When they were seven months old the cheese inoculated with *Streptococcus X* and *S. kefir* was pronounced by experts to be a good Cheddar cheese, with the typical flavor highly developed, whereas the control cheese inoculated with *S. lacticus* alone had a mild, rather pleasing flavor, which did not in any way resemble Cheddar.

In the last series of experiments 10 cheeses were made. One control was made of raw milk with a starter of *S. lacticus*, three were made of pasteurized milk with a starter of *S. lacticus*, to serve also as controls; and six were made of pasteurized milk with a starter of *S. lacticus* and of cheese streptococci. Three strains of cheese streptococci were used in these experiments; one of *Streptococcus X*, one of *S. kefir*, and one whose characteristics did not conform to either of these species; it is not described in this paper because it is an odd strain in the collection of cheese streptococci. Its beneficial effect when inoculated into pasteurized-milk cheese merits for it further study and a determination of its frequency in dairy products. At the time of this writing the cheeses have been ripening for six weeks at a temperature of from 13° to 15° C. The raw-milk cheese is unquestionably the poorest of the lot. There are many small gas holes in it, and the flavor is unclean. It was made during very hot weather, and the milk was poor in quality, as is usually the case under such conditions of temperature. All three of the cheeses made of pasteurized milk and inoculated with *S. lacticus* are good in texture, and have the clean, acid flavor typical of cheeses made in this way. All three strains of cheese streptococci, when inoculated singly or in various combinations, improved the flavor of the cheese as compared with the controls. The acid flavor characteristic of the control pasteurized-milk cheese is lacking; instead there is a mild

and more delicate flavor, unanimously preferred to the acid flavor by the four cheese experts to whom the samples were submitted. The curd is also more broken down in several of the cheeses inoculated with cheese streptococci, which indicates a more rapid ripening.

The experiments have shown that the cheese streptococci improve the flavor of cheese made of pasteurized milk according to the Cheddar process. The cheeses were made on an experimental scale, where there was every facility for handling the pure cultures, but it is hoped that practical methods may be devised whereby any manufacturer can make use of these pure cultures in his factory.

Sammis and Bruhn (*18*) worked out the method by which cheese of the Cheddar type can be manufactured from pasteurized milk, and they pointed out the advantages of pasteurizing the milk for making that kind of cheese. They sent to city markets great quantities of their cheese which had been inoculated with *S. lacticus* alone. From the reports of the dealers they conclude:

> There appears to be no reason why pasteurized-milk cheese cannot be sold regularly in any market, with entire satisfaction, excepting possibly, to the limited trade that demands very high flavored cheese.

The experiments recorded in this paper show that the pasteurized-milk cheese may not only be improved in flavor by inoculation with a starter of cheese streptococci, but also the time of curing may be shortened, an important consideration with the cheese manufacturer. When the makers of Cheddar cheese recognize the advantages of pasteurizing the milk, there is no reason why a practical method may not be developed by which any manufacturer could make a choice of the strain of streptococcus or the combination of streptococci which would give a flavor he desires, and by using it constantly under uniform conditions the whole output of a factory could be made to develop its own "trade" flavor.

As a result of this and earlier (*8*) work, it may be concluded that the cheese streptococci are essential factors in normal Cheddar cheese ripening. A few fundamental biochemical reactions have been determined but not enough is known of these streptococci to explain fully the chemical processes they may be responsible for in the cheese ripening. A few facts are suggestive, however, of the part the several types of streptococci may play in the Cheddar cheese ripening. The formation of large quantities of acetic acid in milk cultures by *Streptococcus X* and *S. kefir* suggests that these organisms may be responsible for the production of alcohols and esters, substances which, according to Suzuki, Hastings, and Hart (*20*) give flavors to Cheddar cheese. They found that acetic acid formed 90 per cent of the acids obtained by the saponification of the esters and the oxidation of the alcohols of their "flavor solution" from ripe Cheddar cheese. As yet it is unknown whether the acetic acid is formed simultaneously with the lactic acid in the decomposition of the

lactose, whether it results from the decomposition of the lactic acid, or whether it comes from protein cleavage.

The vigorous production of carbon dioxid by cultures of *S. kefir* explains the hitherto unaccounted-for production of large quantities of that gas in Cheddar cheese. Van Slyke and Hart (*22*) found that a normal Cheddar cheese during 32 weeks' ripening produced carbon dioxid equal to 0.5 per cent of the fresh cheese. They found that carbon-dioxid production in the cheese continued for months after the disappearance of the lactose, and they state that apparently it came from reactions taking place in some of the amido compounds. The source of the carbon dioxid has not yet been determined, but from our results also it seems improbable that it came from a decomposition of the lactose, since a vigorous decomposition of the latter indicated by the increase in hydrogen-ion concentration takes place in peptone-broth cultures, with the production of a slight quantity of gas, whereas in digested milk an abundant gas production accompanies the lactose fermentation.

The results of cultural studies indicate that in peptone-yeast broth free of fermentable substances, the protein cleavage takes place to exactly the same extent in cultures of *S. lacticus* and *Streptococcus X*. Nevertheless the two organisms do not bring about the same changes in the casein of ripening Cheddar cheese. It has been mentioned above that the pasteurized-milk cheese ripened by *S. lacticus* alone has an acid flavor, whereas a cheese made in the same manner but with the use of a starter of cheese streptococci in addition to the *S. lacticus* starter develops a milder flavor. To judge by the palate, the difference in flavor is pronounced, but the difference in terms of hydrogen-ion concentration is slight though definite. Two pasteurized-milk Cheddar cheeses inoculated with *S. lacticus* alone after about 10 weeks' ripening had a hydrogen-ion concentration of P_H=5.7. A cheese made at the same time and inoculated with *S. lacticus* and *Streptococcus X* had a hydrogen-ion concentration of P_H=5.9, and a fourth cheese inoculated with *S. lacticus*, *Streptococcus X*, and *S. kefir* also had a hydrogen-ion concentration of P_H=5.9. This slight difference of 0.2 P_H may have been responsible for the difference in protein cleavage, which was evident in the cheeses in the white, opaque, somewhat soggy mass in the case of those inoculated with *S. lacticus* alone, as compared with a more translucent, yellowish mass in other cheeses.

CHEESE STREPTOCOCCI IN SOFT-CREAM CHEESE

The case is very much the same with soft-cream cheese as with Cheddar. Cream cheese made from the best quality of raw milk develops a mild, delicate flavor typical of this kind of cheese. Cream cheese made from pasteurized milk and inoculated with a starter of *S. lacticus* develops an agreeable acid flavor, particularly liked by many people, but on the whole is inferior to the delicate flavors developed in cream cheese made

from the best quality of raw milk. As in the case of Cheddar, cream cheese made from pasteurized milk is whiter than that made from raw milk. Anyone who has the opportunity to compare cream cheese made according to the different methods learns to distinguish them quite readily by their flavor and color. The texture also differs slightly.

Experimental soft-cream cheeses have been made from pasteurized milk with the use of *Streptococcus X* and *S. kefir* alone or together, in addition to the *S. lacticus* starter. The results were definite, showing that flavors in pasteurized-milk cheese can be influenced at will by a choice of streptococci in starters. A cream cheese inoculated with *S. lacticus* alone has its own peculiar flavor; a cream cheese inoculated with *S. lacticus* and *Streptococcus X* has a decidedly different flavor; another inoculated with *S. lacticus* and *S. kefir* has another flavor; and when all three species of streptococci are used together, still another flavor is produced. And as some people prefer their ice cream flavored with vanilla, and others like chocolate flavoring best, so the one cheese flavor is more pleasing to some people, while others prefer another. The pasteurization of the milk assures a uniform product in cheese making. This is one reason why manufacturers are loath to adopt the pasteurization of milk for cream-cheese making. They want to develop the cheese flavors which they believe to be peculiar to their own product. It may be that certain strains of streptococci have gained predominance in certain localities, so that season after season a manufacturer of cream cheese can reproduce his own peculiar cheese flavors. The experiments have demonstrated that it will be possible for a manufacturer to choose the flavor which he may want his product to develop in cream cheese made from pasteurized milk. Whether the consuming public will be discriminating enough to make the inoculation of soft-cream cheese with cheese streptococci a financial success remains to be demonstrated.

SUMMARY

(1) Streptococci that differ from *S. lacticus* are common in ripening cheese of various kinds, and in other foods prepared by fermentation. In this paper they are called cheese streptococci. It is probable that a study of the mouth, fecal, and udder types of streptococci will show that cheese streptococci belong to those familiar types.

(2) *S. lacticus* is described culturally and biochemically, and two other species of streptococci, *Streptococcus X* and *S. kefir*, are likewise described.

(3) The most pronounced biochemical characteristic which distinguishes *S. lacticus* from the other two species of streptococci described is the small quantity of acetic acid which it produces in milk cultures.

(4) *S. kefir* is notable among dairy streptococci because of its vigorous production of carbon dioxid when grown in suitable media.

(5) The experiments demonstrate that cheese streptococci modify significantly the flavor of pasteurized-milk cheese. *Streptococcus X* and *S. kefir* and another unclassified strain improved the flavor and hastened the softening of the curd of cheese made according to the Cheddar type from pasteurized milk. *S. kefir* and *Ss. X* also gave distinctive flavors to soft-cream cheese made of pasteurized milk.

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INTUMESCENCES, WITH A NOTE ON MECHANICAL INJURY AS A CAUSE OF THEIR DEVELOPMENT[1]

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INTRODUCTION

Little attention has been given by plant pathologists to the pustule-like outgrowths of tissue to which the appellation "intumescences" may be appropriately applied. The accounts of them deal mainly with descriptions of their occurrence upon the leaves, flowers, fruits, and twigs of various species of plants and with suggestions regarding the cause, in most cases not supported by experiments. Since no great imaginative power is required to see that the problem of intumescences is merely a part of the greater problem of all excrescences or overgrowths in plant and animal tissues, such as galls, tumors, knots, cankers, edemata, crowngalls, etc., intumescences then become of immediate interest to both plant and animal pathologists. While the remote or ultimate causes of these overgrowths may be entirely different, investigators of these phenomena do not appear to realize that they are probably dealing with the same proximate cause. This fact is illustrated by studies on insect galls, concerning which an enormous volume of literature has accumulated; on crowngalls, our knowledge of which is due largely to the illuminating researches of Smith (*9*, *10*, *11*)[2] and his associates; and on edemata, the cause of which is so clearly demonstrated in the brilliant experimentation of Fischer (*3*). It is the present purpose, therefore, to review briefly the more important contributions dealing with the cause of intumescences, to describe intumescences arising from mechanical injury, and to suggest that their proximate cause is the same as of edema in animals, the explanation of which appears to have been generally overlooked by plant pathologists or has not been regarded by them as applicable to overgrowths in plants.

HISTORICAL REVIEW

The designation "intumescentia" appears to have been first applied by Sorauer (*12*, *p. 222*), to pustular processes, generally yellow in color, formed upon the surface of leaves. The cells which make up these processes are always more or less enlarged. In explanation of their occurrence he states that they are brought about by such conditions as

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[2] Reference is made by number (italic) to "Literature cited," p. 238–239.

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excessive moisture (*13*) or abnormally high temperature (*14*), coordinated with a reduced assimilatory activity occasioned by weak illumination. The same factors are employed by various other investigators, including Atkinson (*1*), Prillieux (*6*), Noack (*5*), Trotter (*16*), and Steiner (*15*), in explanation of the cause of intumescences.

In contrast with this explanation is that of Viala and Pacottet (*17*) and Dale (*2*), who maintain that brilliant illumination, together with a moist atmosphere, is to be regarded as the ultimate cause.

Küster (*4*) expressed the opinion that the introduction of poisonous substances may be a cause of intumescences and that they are manifestly related to insect galls. The poison for the development of these galls, he believed, was produced by the gall-forming insect. A review of literature upon gall formation, a matter beside the present purpose, and a rejection of the theory that galls result from the injection of a chemical substance by the insect, are contained in a recent paper by Rosen (*7*).

The production of intumescences upon cauliflower as a result of the stimulatory activity of copper compounds applied as sprays has been demonstrated by Von Schrenk (*8*) and has subsequently been confirmed by Rosen (*7*) and by Smith (*11*). In explaining their proximate cause, Von Schrenk maintains that the stimulatory activity is probably due to high osmotic tensions resulting from compounds of the copper salts with the protoplast.

Smith's concept of overgrowths, particularly of crowngall and malignant tumors, as expressed in recent papers (*10*, *11*), is that they are due to the removal of normal growth inhibitions. He found that various substances—namely, aldehyde, acetone, alcohol, acids, and alkalies—removed these inhibitions, not by direct chemical action, however, but by locally increasing the osmotic pressure; hence, by physical action. In the case of crowngall, various soluble substances were found to result from the metabolism of the parasite in culture. If, as he contends, these substances are slowly and continuously supplied by the intracellular growth of the organism, they would lead to osmotic disturbances which would tend to be equalized by the movement of water and dissolved foodstuffs toward the parasitized cells.

Fischer (*3*), to whom reference has previously been made, relates the cause of swellings of animal tissues which manifest themselves in states known as edema, glaucoma, and nephritis to the phenomenon of absorption both of water and of dissolved substances. The cell colloids and their state, according to this theory, determine the amount of water held by a cell, a tissue, an organ, or even the entire individual under different physiological and pathological conditions. The cause, then, of normal absorption, and consequently of abnormal absorption, resides within the cells themselves. The nature and cause of edema can most

succinctly be presented by quoting the author's terse summary, in which he says (p. 190–191):

A state of œdema is induced whenever, in the presence of an adequate supply of water, the capacity of the colloids of the tissues for holding water is increased above that which we are pleased to call normal. Any agency capable under conditions existing in the body, of thus increasing the hydration capacity of the tissue colloids constitutes a cause of œdema. The accumulation of acids within the tissues brought about either through their abnormal production, or through the inadequate removal of such as some consider normally produced in the tissues, is chiefly responsible for this increase in the hydration capacity of the colloids, though the possibility of explaining at least some of it through the production or accumulation of substance (of the type of urea, pyridin, certain amins, etc.) which can hydrate colloids as can acids, or through the conversion of colloids having but little capacity for water into such as have a greater capacity must also be borne in mind.

DESCRIPTION OF INTUMESCENCES FOLLOWING INJURY

During the early part of June, 1917, a windstorm of sufficient velocity to break down trees of considerable size occurred in the vicinity of Raleigh, N. C. A few days later, the writer, on a field trip into the sandy lands east of the city, noted that the leaf surface of cabbage (*Brassica oleracea capitata*), particularly the lowermost leaves and the tips of the inner leaves, were covered with numerous irregularly disposed, wartlike prominences (Pl. 18, A). The individual outgrowths were hemispherical to short cylindrical in shape, yellowish to grayish in color, and projected from the surface of the leaf in a sharply defined circle. They ranged in size from mere points to structures having a diameter of 3 mm. and in extreme cases projected from the leaf surface as much as twice the thickness of the cabbage leaf. A depression corresponding to the prominence commonly appeared on the opposite leaf surface. In some places the outgrowths were so numerous as to merge into each other and consequently appeared like one large, irregular intumescence.

Microscopic examination of these intumescences shows that they begin as small swellings of the epidermis. Soon the palisade parenchyma beneath, or the spongy parenchyma, depending upon whether the intumescence originates on the upper or lower leaf surface, is involved, and the cells begin to become enlarged. At this stage the epidermis is broken or elevated on the surface of the growing projection. Some of the epidermal cells then shrivel and dry and remain as fragments on the surface of the intumescence, while others are included in the intact swollen tissues. Meanwhile the cells beneath have continued to enlarge and all of the subjacent mesophyll comes to be involved in the formation of the edematous cells. These cells are enlarged to many times their normal volume (fig. 1), are elongated, very thin-walled, and are practically destitute of chloroplasts and other protoplasmic contents. There appears to be no tendency toward suberization, as is indicated by negative tests with alkannin. The cells are not to be regarded as giant cells in

the sense in which this term is applied by animal pathologists in that they are not multinucleate, but remain uninucleate. The yellowish or whitish appearance of the protrusion is accounted for by the fact that the intercellular spaces are filled with air.

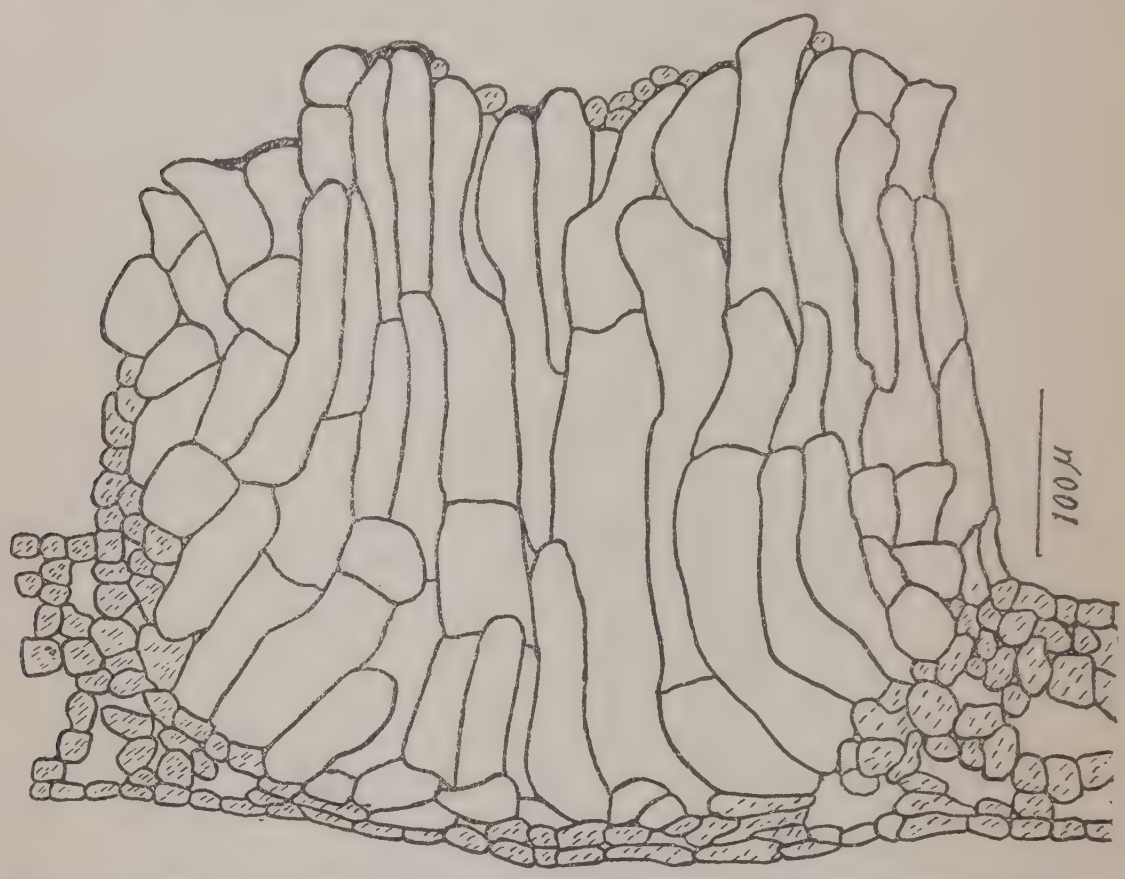

FIG. 1.—Outline drawing, made with the aid of a camera lucida, of a vertical section of an intumescence on cabbage.

Since neither fungi nor bacteria were associated with these intumescences, it was conceived that they must have resulted from injuries inflicted by wind-blown sand. This deduction was subsequently confirmed both under field and greenhouse conditions by violently projecting sand against normal cabbage plants. Intumescences of the type shown in figure B of Plate 18 developed within four to six days following the wounding. The intumescences thus artificially produced were identical in appearance and structure with those first found. They could not be made to appear upon mature parts, but only upon actively growing tissues. They occurred both on the lamina of the leaf and upon the veins. In the latter case the xylem elements were not modified, the only modification occurring in the parenchymatous cells surrounding the vascular bundles. Sections through intumescences upon small veins showed that the normal vascular elements may be entirely surrounded by hypertrophied cells.

Humidity was observed to operate in conjunction with the age of the tissues in the development of intumescences. When a plant injured by sand cast upon it was covered with a bell jar in order to maintain a high relative humidity, the edematous cells continued to enlarge until the

pustule projected from the leaf surface to a height equal to three or more times the thickness of the leaf (Pl. 19). When the enlarged cells were exposed to desiccation, however, the protrusions were much less prominent, as would be anticipated. Humidity had previously been reported by the writer as exerting a similar effect in the production of cankers on *Citrus* spp. (*18*).

DISCUSSION OF RESULTS

It must be admitted that the development of intumescences upon young cabbages following the violent projection of sand against them came somewhat as a surprise, even though wind-blown sand was suggested to the grower as the probable cause when the field diagnosis was made. Mechanical injury appears not to have been previously observed to be an ultimate cause of the formation of intumescences on plants. The complete chain of events connecting the stimulus of wounding at the one end and the mature intumescence at the other is, of course, not completely known. However, it is believed, in the light of the theory of edema as advanced by Fischer (*3*), that an adequate explanation can be offered for the formation of the enlarged cells. The exposure of the protoplasts following the rupture of the epidermis from the impact of the particles of sand would certainly be followed by oxidative changes within these protoplasts. This assumption is based on the fact that Von Schrenk (*8*) found an increase in the oxidizing enzyms in intumescences on cauliflower (*Brassica oleracea botrytis*) leaves and upon our general knowledge of oxidations following exposure of tissues through wounding. The acids produced from these oxidations would not only increase the hydration capacity of the emulsion colloids within the wounded cells but would also involve adjacent cells. The fact of a heightened affinity of colloids for water following even a slight increase in acidity is a well-known property of colloids. This would be followed by the distention of involved cells to a point where equilibrium had been established, a condition determined by such factors as differences in adsorption, solubility, and chemical combination of the cell colloids themselves.

This explanation, while it may not meet with approval by students of overgrowths, is attractive because of its simplicity and because it accords with established facts concerning absorption by hydrophylic colloids. It is furthermore believed that the theory of absorption can be applied to the experimentation presented by other investigators and can also be employed to explain adequately the mechanism of other types of overgrowths in plants such as knots, tumors, and galls of insect, nematode, bacterial, and fungus origin.

SUMMARY

(1) Various ultimate and proximate causes have been assigned for the formation of different types of overgrowths as intumescences, cankers, knots, tumors, and galls present on plant parts.

(2) Wind-blown sand as an ultimate cause of the formation of intumescences on cabbage appears not to have been previously reported.

(3) The proximate cause of these intumescences is believed to be a problem of absorption and is due to a heightened hydration capacity of the cell colloids resulting from acids produced by oxidation.

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PLATE 18

A.—Intumescence on cabbage formed as a result of injury from wind-blown sand.

B.—Intumescences produced following injury from sand artificially projected against the leaves of cabbage.

A

B

A

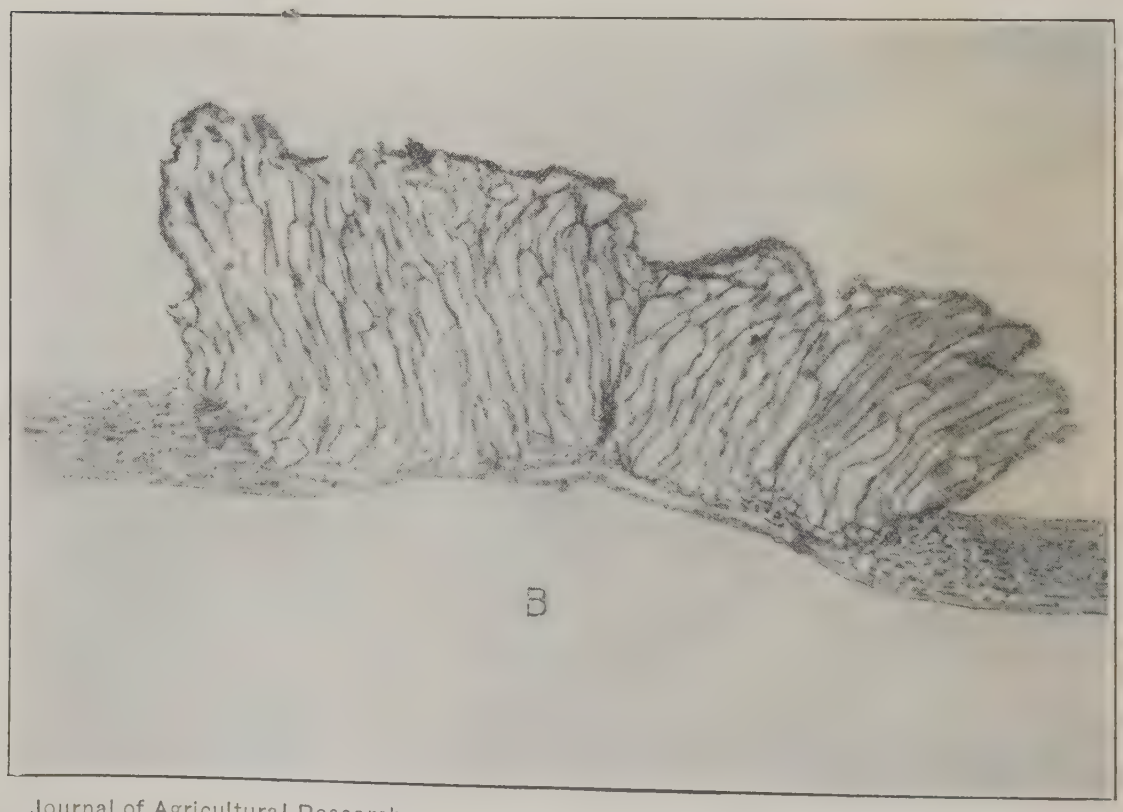
B

PLATE 19

A.—Photomicrograph of a small columnar intumescence on cabbage, following artificial injury.

B.—Photomicrograph of a large cushion-like intumescence developed after artificial injury.

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ANTHRACNOSE OF LETTUCE CAUSED BY MARSSONINA PANATTONIANA

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INTRODUCTION

Lettuce (*Lactuca sativa*) is not an easy crop to grow successfully, because of the peculiarities of the plant, its quick response to unfavorable conditions, and its susceptibility to disease. Any disfigurement of the leaves renders it unmarketable. In this regard it is rather unique, for most other food plants may be attacked in some part by insects or fungi, and still may not be a total loss.

The disease considered in this paper does its chief damage to greenhouse lettuce. The methods of culture under glass are very important factors in determining the extent of damage from this disease. In general, several crops are grown during the winter season. Seeds are sown very thickly in beds, usually in raised benches in a greenhouse devoted to the production of seedlings. After one or more transplantings the small plants, 3 or 4 inches tall, are set out with 6-inch spacings in beds in the main greenhouse. The soil is heavily enriched with manure or other fertilizer. The watering is done either with hose or, in the better greenhouses, with an overhead sprinkling system. The temperature is kept about 55° F.; and with the best growers ventilation is maintained practically all of the time, excepting, of course, the extremely cold nights.

Seed beds are planted so that a succession of crops can be raised. Although some head lettuce is grown, by far the bulk of the crop is of the leaf-lettuce type. The crop is harvested when the plants weigh from 3 to 6 ounces, the state of the market and the maturity of the lettuce being governing factors. The bunches are trimmed as harvested, the débris being turned under as a general rule.

Naturally with such intensive methods as the heavy investment in glass and equipment demands, many parasitic diseases become im-

[1] This work was done at the suggestion of and along lines outlined by Dr. G. H. Coons, of the Michigan Agricultural College, and I am indebted to him for help in the preparation of the manuscript. I also wish to thank Dr. E. A. Bessey, Professor of Botany in the Michigan Agricultural College, for kindly assistance and encouragement during the course of the work.

portant. The general effects of these troubles may be contrasted with the anthracnose. Aside from the insect and nematode depredations, we have in general two types of injury to the mature plants; diseases which rot the plants more or less, and those which disfigure the leaves. In the first group may be mentioned drop, caused by *Sclerotinia libertiana*, and graymold, caused by *Botrytis* sp. Occasionally the softrot caused by *Bacillus carotovorus* causes damage. Frequently lesions are found in the petioles and blade which lead to the rotting off of the other leaves of the plant. This disease is called "black stemrot," or "bottom-rot," and species of Rhizoctonia have been found associated with this trouble.

Of the leaf diseases, the downy-mildew, caused by *Bremia lactuca*, and the anthracnose are the most important. The effects of *B. lactuca* are such as would be expected from one of the downy-mildews. Light-colored areas are noted on the upper surface of the leaves, while the lower surface shows the conspicuous tufts of conidiospores of the organism. With young plants a drying up of the leaves may occur.

COMMON NAME OF THE DISEASE

In the previous accounts of this disease (*3*),[1] several common names have been used: "anthracnose," "shothole," "leaf-perforation," and "rust." In Michigan greenhouses the name "rust" is probably most common. The causal organism, however, has no connection with the true rusts of plants. Hence, this name, though fairly descriptive and popular, is, according to the prevailing usage among pathologists, not to be recommended. The names "shothole" and "leaf-perforation," while very appropriate, in so far as the lesions on the leaf blade are concerned, do not properly consider the effect on the midrib, which is by far the more disfiguring phase of the trouble. The name least open to objection, and the one which is in accord with the best usage among plant pathologists, is "anthracnose." This term has become a common word among growers of other crops affected by similar parasitic diseases, and will no doubt be readily accepted by greenhouse men.

PREVIOUS INVESTIGATIONS

The first account of the anthracnose of lettuce and the associated organism was given by Berlese (*2*) in 1895. This writer, in a short note after a description of the signs of the malady, named the associated organism "*Marssonia panattoniana*." As will be seen in the discussion of the etiology of this disease, Berlese recognized the parasitic nature of the fungus, but did not make conclusive inoculation experiments.

At almost the same time Selby (*8, p. 224*) published a brief note in which he described the appearance of the affected plants. The associated fungus was named *Marssonia perforans* E. and E., Ellis and Ever-

[1] Reference is made by number (italic) to "Literature cited," p. 280.

hart having determined the fungus from Selby's collections. The fungus was reported as being quite prevalent in Ohio greenhouses that year and as causing considerable loss to lettuce growers. In a succeeding publication Selby (*9*) repeated the statements of former bulletins and stated that the fungus had since been discovered at other points in the United States.

No doubt the most important contribution to the literature of this disease was that of Appel and Laibach in 1908 (*1*). In the Province of Brandenburg, Germany, an epidemic caused by the fungus called *Marssonia panationiana* in the spring of 1907 caused many growers to destroy thousands of plants. It is noteworthy that the fungus appeared in the open, while American observations dealt with the disease in greenhouse plants. Appel and Laibach made important notes on the morphology of the fungus, grew it in pure culture, and made inoculation experiments with spores from the diseased host.

Dandeno in 1907 (*4*) from examinations of Michigan material came to the conclusion that the fungus, called by him "*Marssonia perforans*," was not properly classified as Marssonia, but was rather a species of Didymaria.

Kirchner, in 1906 (*6, p. 376, 389*), without citing his authority, records the disease from Holland and Italy on endive, as well as lettuce.

Aside from repetition of statements from former literature in the general handbooks of plant diseases, no other publication on this disease or its causal organism has come to the writer's attention.

DISTRIBUTION OF THE FUNGUS

The distribution of the fungus is no doubt wide. No record exists of its presence in Europe, except in Germany, Holland, and Italy. Meagerness of accounts may be responsible for this lack of record. From the notes in the literature, the fungus is known from Ohio and Michigan. The Ohio records date from 1895. The first Michigan specimens were collected on March 20, 1897, at Grand Rapids, by B. O. Longyear. Since then the fungus has been found repeatedly at various parts of the State. During an excessively wet June in 1916 it was found at East Lansing as a serious pest on lettuce grown in the garden.[1] Early in 1917 it was observed by the writer in a greenhouse at Ithaca, N. Y., where 5 to 10 per cent of the plants were a total loss, and many of the remainder were so badly affected as to require the stripping of the outer leaves. Since that time it has been reported from various parts of New York State by Mr. H. W. Dye, Dr. Charles Chupp, and Dr. I. C. Jagger. Dr. Jagger informed the writer that he had noticed the disease in previous years in New York State.

[1] The writer is indebted to Dr. G. H. Coons for this report.

The records of the Plant Disease Survey of the United States Department of Agriculture are as follows:

No date. Michigan (R. H. Pettit).[1]
Caused loss of thousands of dollars in the forcing houses.

1910. Ohio (A. D. Selby).
Reported in one county.

1912. Oregon (H. S. Jackson).
Limited.

Utah (C. N. Jensen).
15 per cent in some greenhouses.

1915. North Carolina (H. R. Fulton).
Loss considerable.

Washington (F. D. Heald and D. C. George).
Occurrence only.

ECONOMIC IMPORTANCE OF ANTHRACNOSE

The disease was stated by Selby (*8*) to be very important in 1895. It is reported to have been quite prevalent in Ohio during that year and to have caused considerable losses to the lettuce growers.

Appel and Laibach (*1*) reported a 50 to 60 per cent loss in the fields about Brandenburg, Germany.

Authoritative reports from growers in Grand Rapids in 1898 and a few years following indicate that the disease was serious enough to cause the loss of most of the crop in practically the entire acreage about Grand Rapids. Since that time the losses have been less uniform, and at the present time only occasional greenhouses show serious outbreaks of the disease. Careful search in almost any greenhouse will reveal occasional plants, but few epidemic outbursts of the disease are now reported.

A plausible explanation of this decrease in the amount of disease is given under the discussion of dissemination of the fungus.

SYMPTOMS OF THE DISEASE

The general effect on the plant in this anthracnose is to produce a general dwarfing and discoloration (see Pl. C). Beds with affected plants have a brown or yellowish appearance. In the field the disease in a moist season may lead to death of the outer leaves. Since the attack is progressive, passing from older to younger inner leaves, the effect is to make plants small and of second grade, if not indeed a total loss. The fungus is found on the leaf blades and on the midribs.

On the leaf.—The fungus produces a characteristic spot on the leaves of affected lettuce plants. The diseased areas show first as small, pinpoint, water-soaked spots. These rapidly enlarge, and soon a straw-colored spot 3 to 4 mm. in diameter is produced (Pl. 20, A). The spot is either circular or angular, depending on the proximity of large veins.

[1] February, 1906 (from records of the Michigan Experiment Station). At this time research by Prof. Pettit and Mr. Moses Craig resulted in recommendations of subirrigation as means of water supply, together with surface applications of sand. No publication was made of work done.

In the last stages the spots show dead areas at the center, and these may fray or drop out, giving the leaf a characteristic shot-hole appearance. Such a leaf appears as if gnawed by insects. Affected leaves wither quickly, and the lettuce is liable to rot under storage conditions. Evidence of spore production is seldom seen with the unaided eye even in cases of severe infection, although spores can always be obtained from such spots. If, however, the diseased leaves are placed under moist conditions, a film of pinkish-white spores appears at the centers of the diseased spots.

On the midrib.—The lesion on the midrib is of the type characteristic of the anthracnoses. Sunken elliptical spots commonly appear in great numbers (Pl. 20, B). These spots vary with the age and the condition of the leaf. They are elongated in the direction of the long axis of the leaf, 4 to 5 by 2 to 2.5 mm., and are not noticeably sunken. This elongation of the spots on the nerve is undoubtedly due to the shape of the cells in that situation, which are very long and narrow, and offer less resistance to the progress of the parasite in a longitudinal than in a transverse direction, on account of the fewer cross walls. The depression is more apparent in these elongated nerve spots than in the round spots on the blade of the leaf, because the tissue of the nerve is thicker and more fleshy, and the collapse of a greater number of cells is possible.

These spots begin as barely noticeable water-soaked areas. After a few days the spots become a straw-yellow, while in age they are reddish yellow. Badly affected midribs present a very irregular contour. With the progress of the disease the functions of the entire leaf are disturbed. Occasionally the attack is severe enough and the lesions deep enough for the weight of the leaf to cause the breaking or bending at the affected point. Badly affected leaves often wither, owing, no doubt, to the loss of water from the broken epidermis.

ETIOLOGY OF THE DISEASE

Since Berlese's first report (2), the etiological relation of *Marssonina panattoniana* (*Marssonia perforans* in American literature) to the disease has been assumed. Berlese, however, performed no experiments to prove the causal relation. The work of Selby was of similar character. Appel and Laibach cultured the organism, but gave no evidence in their article of having used pure cultures in inoculation experiments. The successful inoculation experiments which they described were those in which spores from diseased plants were transferred to healthy plants.

Proof, therefore, of the etiology of the anthracnose of lettuce depends entirely upon constant association of the host and organism along with evidence of the infectious nature of the disease. Although this is no doubt sufficient in the case of the organisms of such known pathogenic habit as the Melanconiales, it is now possible to complete the proof.

Diseased lettuce leaves were collected from a greenhouse at Grand Rapids on January 16, 1915. Some of the material was placed in moist chambers where it remained until February 20. A microscopic examination showed an abundance of typical spores. A large drop of water was placed on the spot. After a short time this water was drawn up into a capillary tube. Plates of prune-juice agar were planted by making streaks across them with the capillary tube. Fungus colonies appeared in abundance and were transferred to test tubes. After two days, sporulation which was recognized as typical had occurred, and plates were poured by the loop dilution method, with prune-juice agar. A single spore was located with a microscope, and a pure culture was thus obtained. This was increased, and the subcultures were used for inoculation. Twelve lettuce plants growing in 6-inch pots in the greenhouse were inoculated by means of punctures along the upper surface of the midribs. Control plants were punctured with a sterile needle. In five days typical lesions of the disease had appeared on the inoculated plants, and the organism was reisolated by the loop dilution method, the spores being lifted from an acervulus by means of a sterile platinum spatula. None of the control plants became diseased. A new single spore culture was thus obtained whose relation to the disease had been established by isolation, inoculation, and reisolation.

In other experiments with pure cultures, typical lesions have been produced by placing material on the uninjured surface of the leaf blades and midribs (Pl. 20, C).

During the course of the work attempts to inoculate species closely related to *Lactuca sativa*—namely, *L. floridana*, *L. scariola*, and *Cichorium intybus*—failed; but inoculations of all varieties of *L. sativa* tested—namely, Black-Seeded Tennis Ball, Prize Head, and Grand Rapids Forcing—were found to be uniformly successful.

INFECTION PHENOMENA

Experiments started on April 1, in which spores from recently isolated cultures were sown on cover glasses which had been smeared with a thin glucose agar and which were subsequently placed over Van Tieghem rings, proved that the time necessary for germination is very short, ranging from four to eight hours, with an average of six hours at room temperature (25° C.). This experiment was continued in order to determine the time necessary for spore formation. This period was found to be very short also, spores being produced from newly formed conidiophores 30 hours after germination of the original spores and being present in great quantities at the end of 40 hours. On April 18, spores from a fresh culture were sown on the leaf of a young lettuce plant, and the pot containing it was placed beside a compound microscope so that the inoculated leaf could be held firmly on the stage. A thin cover glass

was placed over the leaf where the spores were sown, and the microscope focused upon the surface of the leaf. The spores being hyalin were somewhat difficult to make out with the cells of the leaf as a background;

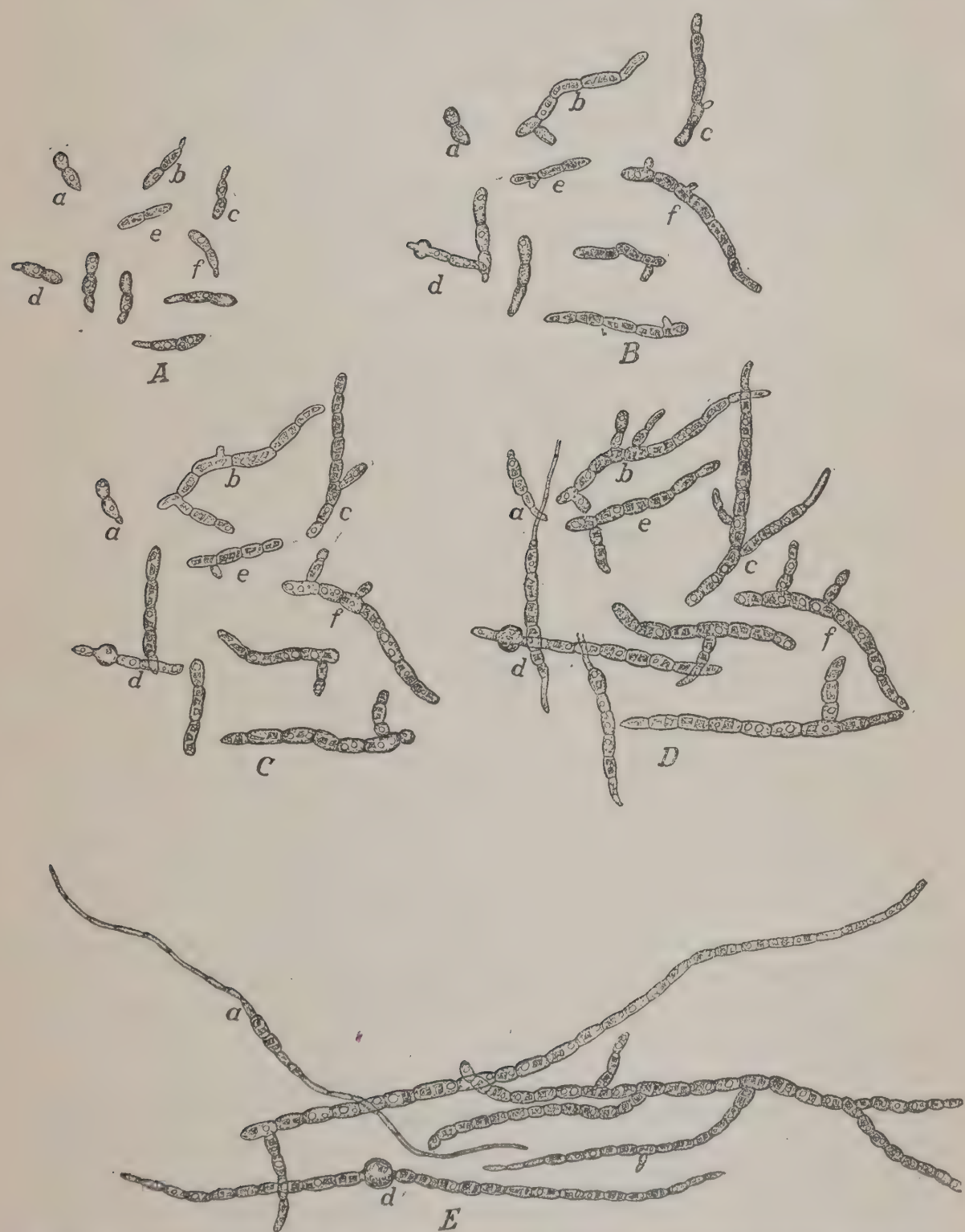

FIG. 1.—*Marssonina panattoniana* (×300): *A*, Germination of spores; *B*, the same spores two hours later; *C*, after four hours; *D*, after six hours; E, after eight hours.

but with a proper adjustment of the diaphragm and condenser, this was accomplished. By this method it was determined that the required period for germination on the host plant is approximately the same as

when grown in culture (about six hours). The germ tube of some of the spores could be seen to enter the cell walls of the epidermal cells. Penetration took place at a point of contact of the epidermal cells; hence, the germ tube probably grew between the cells throughout. In no case was the germ tube observed within a cell (fig. 1, D, showing penetration). In order to determine whether penetration had actually taken place, water was introduced under the cover glass by means of a capillary tube. In the slight currents which were set up, the free ends of the spores whose germ tubes had penetrated swayed back and forth, but did not wash away, as did those whose germ tubes had not entered the host tissue.

RELATION OF TEMPERATURE TO INFECTION

As the season progressed it was found increasingly difficult to infect plants in the greenhouse, and after June 1 this was quite impossible. The assumption that temperature had considerable influence on the ability of the fungus to infect healthy plants was proved by the following experiment.

Twenty-five plants were inoculated from a 22-day-old culture, by smearing mycelium on the upper and lower surfaces of the leaves. Ten plants were left uncovered in the greenhouse, and 10 were covered with bell jars. The temperature of the greenhouse varied from 25° to 38° C. Five plants were placed in a low-temperature thermostat at 15°. The air conditions in the thermostat were humid, being comparable to the plants under the bell jars.

After three days the plants were examined. Those in the greenhouse were apparently unaffected, but the plants which had been placed in the refrigerator had developed the characteristic lesions of *M. panattoniana*. The greenhouse plants were subsequently watched for 10 days, but no infection could be observed. Evidently temperature is an important factor in the inception of this disease. These results indicate that infection periods occur in cool weather rather than in hot, bright weather. This is in accordance with the observations of Appel and Laibach (*1*), who say:

> . . . It may happen in warm dry weather that the attacked leaves fall and yet the plants in spite of that fact go on to the formation of usable lettuce. Damp weather is especially favorable to the development of the fungus, and therefore it is clear that due to this cold wet spring (1907) the disease has taken on such a threatening character.—TRANSLATION.

RELATION OF MOISTURE TO DEVELOPMENT OF LESIONS

Twenty plants were inoculated and placed in a Wardian case equipped with a fine jet for water adjusted to form a spray. A tested[1] hair hygrometer registered 70 per cent. The saturated plants were allowed to remain

[1] Compared with sling psychrometer.

for a week, after which they were removed. The spray was then cut off, and after normal greenhouse conditions had been restored, another set of 20 plants was inoculated and placed in the case for the same length of time. The ordinary greenhouse temperature (65° to 75° F.) prevailed during the entire experiment. No appreciable difference could be observed in the rapidity with which the fungus spread through the leaf in the two cases compared. Apparently, once the infection occurs, it is quite as easy for this fungus to spread within the host under ordinary conditions as under humid conditions. Aside, therefore, from the relation of water to dissemination and germination, it is evident that temperature plays a more important rôle than the moisture relations of the host.

DISSEMINATION OF THE DISEASE

The source of this trouble in greenhouses is somewhat uncertain. The disease was noticed in 1916 by Dr. G. H. Coons in an isolated planting of lettuce on new ground which had never borne a crop before. It has occurred suddenly in greenhouses where no previous outbreaks were known. This indicates the possibility of seed transference of the fungus, or else the existence of the fungus as a soil saprophyte. The experiments reported under "Physiological relations of the causal organism" showed that the spores were not able to survive drying on glass for five days. They might, however, be more resistant in the mucilaginous coat of lettuce seeds. The fungus might also be transported in bits of trash carried with the seed. The presence of the fungus in the seed or in the soil has not as yet been demonstrated.

Natural infection proceeds from the outer, lower leaves to the inner ones, so there is good ground for assuming that the common source of infection in greenhouses is the soil, or rather diseased trash, which is left to overwinter in the ground. Additional evidence to support this view is that diseased leaves which are exposed in the field, packed in sand in cans from January 20 until April 1, were found to contain viable spores. The fungus spreads to the inner leaves either by local infection or by the actual penetration of the mycelium. Upon stripping a leaf from the head it can be seen that the newly infected areas of the inner leaves are adjacent to the old spots on the outer leaves. The disease thus spreads step by step inward until the whole plant is affected. Spots high up on the leaves come from infection by spores. A careful examination of leaves at the early stage of infection reveals minute discolored spots at a considerable distance from diseased areas. By the aid of the microscope a spore can usually be found on the surface of these minute spots, with its germ tube boring into the tissues between the cells. During a dashing rain these spores are possibly splashed high up on the plant from old spots which have begun to sporulate; or they may be carried by some of the many insects which infest the lettuce plant. The fact before mentioned, that the spots are more numerous in the lower part of

the leaf, may be explained by the tendency of the rain water to run down and accumulate in the axils of the leaves, washing the spores with it.

The appearance and location of the newly infected spots on the leaves led to the belief that the dashing rains in the field and the use of hose for watering or syringing in the greenhouses were accountable for the spread of the disease from plant to plant. The following experiment was performed to determine the relation of the splashing of water from plant to plant to the dissemination of the fungus.

Two plots of lettuce in a bench were separated by a wooden partition covered with tar paper, and the rows parallel to and nearest the walk were inoculated with *M. panattoniana* in wounds made with a sterile

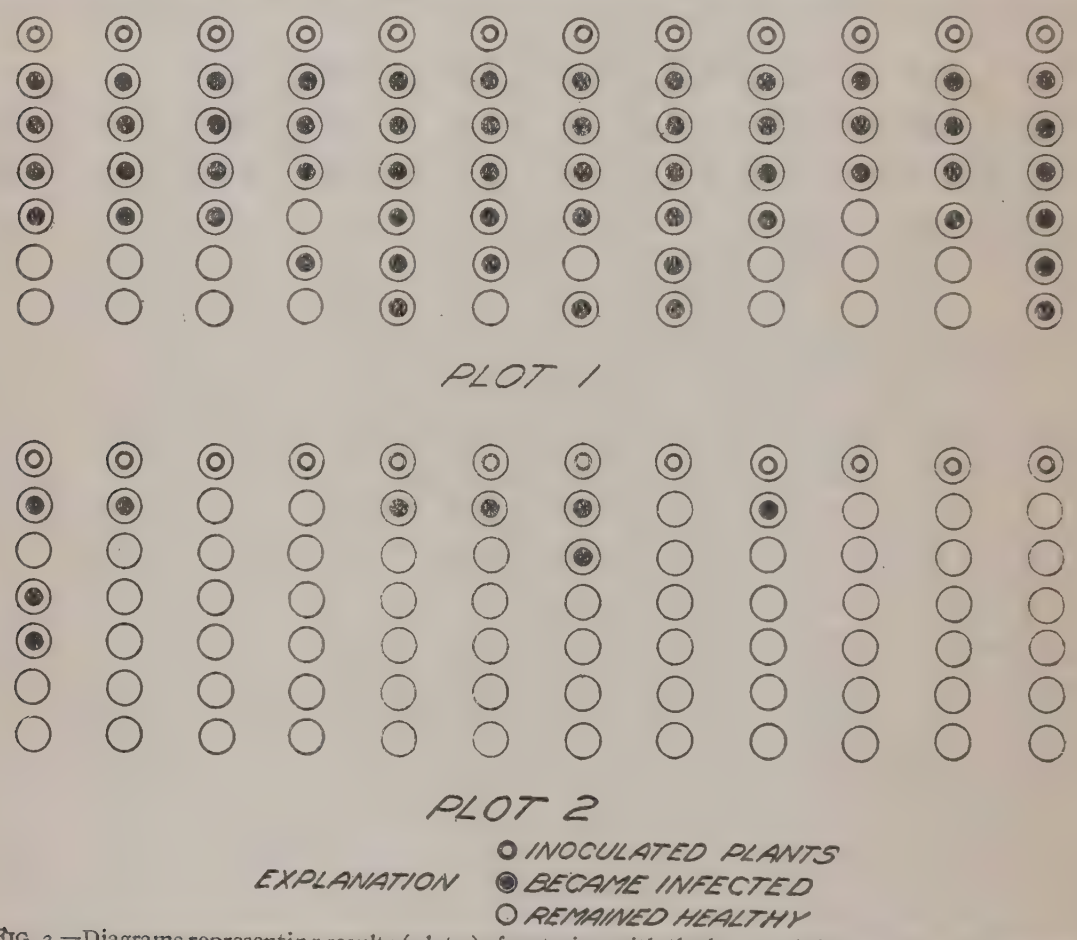

FIG. 2.—Diagrams representing results (plot 1) of watering with the hose and (plot 2) by subirrigation.

spatula. Plot 1 was watered by spraying with the hose. In plot 2 a trench was dug between each row, transverse with the length of the bench, and covered with a long flat board. A notch was cut in each of these boards at the end nearest the walk, and the plants were watered by inserting the hose through this notch and filling the trench with water. The surface of the ground and the plants above ground were thus kept dry during the watering process.

In the plot which was watered by spraying with a hose 55 of the 72 plants were infected in one week. In the plot watered by subirrigation only 9 plants out of the total 72 had been infected in the same period. The results of this experiment are shown very clearly in the accompanying diagrams (fig. 2). The objection may be raised that it was the wet-

ness of the leaves and not the splashing that was responsible for the spread of the disease in this case. Undoubtedly a wet leaf is a more suitable medium for most spores than a dry one, but the wetness of the leaf is of no avail when the mechanism for bringing a spore to it is lacking. It may be mentioned here that in greenhouses, where water drips upon diseased and healthy plants, it has been observed that the plants in the vicinity of the diseased plant become infected, owing to the splashing of drops of water containing spores from diseased to healthy leaves. When water drips on healthy plants alone, the leaves are equally wet, but no infection occurs, indicating that wetness of the leaves alone is not responsible. It is clear that the splashing of water contributes quite largely to the spread of the pathogene in the greenhouse, and probably rain is responsible to a large extent for its spread in the fields. The relation of this to the control of the disease will be considered later.

NAME OF THE PATHOGENE

The organism seems to be properly placed among the Melanconiales. In America the fungus is commonly called "*Marssonia perforans*." The description of this organism fits very well the Michigan specimens. However, Berlese (*2*) described what is undoubtedly the same organism several months previous to the American description. Accordingly the specific name "*panattoniana*" has priority. This was recognized by Appel and Laibach (*1*) who retained the name "*Marssonia panattoniana*."

Mangus (7) has shown that the genus Marssonia (sometimes erroneously spelled "Marsonia") was given in 1861 by Karsten to a Phanerogamic genus. This antedates Marssonia Fischer as a genus of fungi by 13 years. Magnus accordingly has proposed the name "Marssonina" for the preempted genus, and has made the new combination *Marssonina panattoniana* (Berlese) Magnus.

The synonyms of this fungus are as follows:

Marssonina panattoniana (Berlese) Magnus
Marsonia panattoniana Berlese, 1895, in Riv. Pat. Veg., v. 3, no. 5/12, p. 342
Marsonia perforans Ell. and Ev., 1896, in Ohio Agr. Exp. Sta. Bul. 73, p. 225.
Didymaria perforans (Ell. and Ev.) Dandeno, 1906, *in* 8th Rpt. Mich. Acad. Sci., p. 47.
Marssonina panattoniana Magnus, 1906, *in* Hedivigia, Bd. 45, Heft 2, p. 88–91.

MORPHOLOGY OF THE CAUSAL ORGANISM

The fungus which causes the anthracnose of lettuce is a typical member of the order Melanconiales. The mycelium of the fungus is composed of slender, hyalin septate threads which penetrate between the cells of the host. This mycelium is very scanty within the diseased spots except as it becomes matted to form a fruiting layer. This fruiting layer is formed beneath and upon the epidermis at a lesion. The fruiting layer is much more diffuse than that of Gloeosporium or Colletotrichum. For this reason Dandeno (*4*) has suggested that the fungus is in reality a species of Didymaria.

Sections through a lesion in which abundant sporulation occurs show acervuli consisting of closely packed, upright conidiophores, 20 to 60 μ tall (fig. 3, E). From the apexes of these conidiophores slightly curved, 1-septate spores are borne. The spores are hyalin, finely granular, and

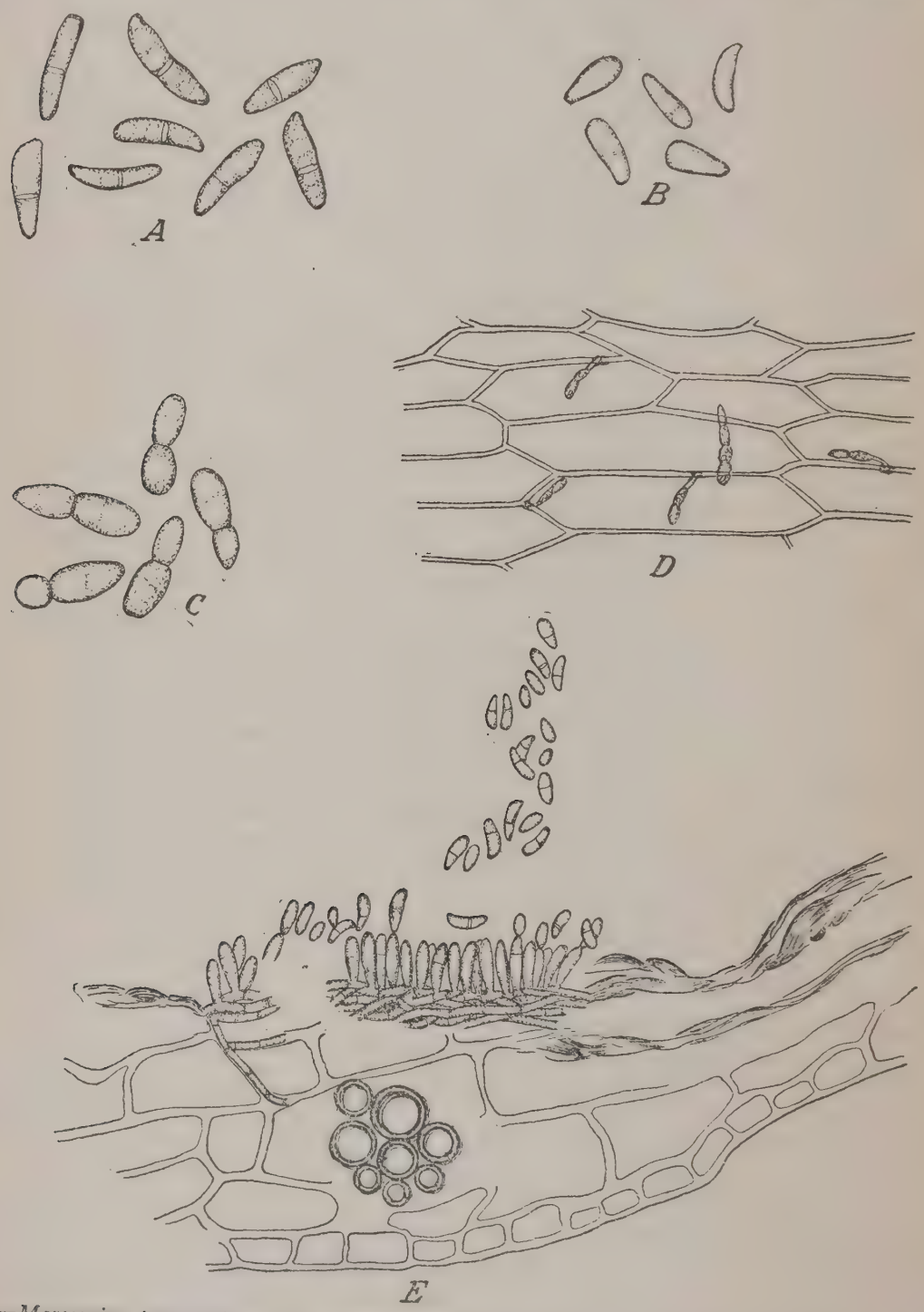

FIG. 3.—*Marssonina panattoniana: A*, Mature spores (× 750); *B*, immature spores (× 750); *C*, spores swollen and constricted at the septa just previous to germination (× 750); *D*, germinating spores on epidermal cells of lettuce leaf, showing method of penetration (× 250); *E*, cross-section of an acervulus drawn from a photomicrograph (× 300).

contain one or two large oil drops. They are constricted at the septum, and the basal cell usually shows a mark where it was attached to the conidiophore. The two cells are about equal in size. The spores average about 4 by 17 μ in size. The immature spores lack the septum and are

much smaller than mature spores. No higher form of fructification is known.

PHYSIOLOGICAL RELATIONS OF THE CAUSAL ORGANISM

GERMINATION PHENOMENA

In order to study the germination phenomena, a suspension of spores in very thin prune-juice agar was prepared, and a loopful of the preparation conveyed to each of a number of cover glasses. These were inverted over Van Tieghem rings and placed on the stage of a compound microscope. After germination had started drawings were made every hour with the aid of a camera lucida to show the progress of the development of individual spores. The smear of agar on the cover glass was purposely made thin enough to prevent the germ tube and later the mycelium from growing out of focus. Complete drawings of the organism up to 40 hours old were obtained in this way. The spores, when first produced, are single-celled, hyalin, granular, with occasional oil drops, ovate or slightly larger at one end, and measure, on the average, 7 to 8 by 2.5 to 3 μ (fig. 3, B). These small immature spores begin to swell up and a cross-wall is formed, dividing the spore into two unequal cells, or less commonly into two equal cells (fig. 3, A). The spore becomes constricted at the septum, and may be slightly curved; but many of them remain straight (fig. 3, C). When completely mature and ready to germinate, the spores measure, on the average, 4 by 17 μ. Individual spores may exceed or fall short of this size by a few microns, but they are fairly uniform in size. When spores are placed in a favorable medium (prune-juice agar was used with good results), development commences at once, the spores enlarge, and in six to eight hours put forth a short, thick germ tube, which apparently may originate at the sides or end of either cell. This quickly enlarges until it is the size of the cell producing it. When it is sufficiently elongated, a cross-wall is formed, cutting off an additional end cell. This in turn elongates, another cross-wall is formed, and thus cell by cell a long strand of mycelium is formed. It may be four or five cells long two hours after germination (fig. 1, B). Sometimes an additional germ tube may arise from the same cell (fig. 1, B, c) or from the other cell of the original 2-celled spore (fig. 1, B, f), which develops in the same manner as the first one. Branches may spring from any of the newly developed middle cells of the chain, and eight hours after germination a considerable ramification is often observed (fig. 1, E). In some cases chlamydospores are formed (fig. 1, B, d), but this phenomenon seems to depend upon the nature of the medium. Thirty hours after germination a dense network of mycelium has developed and numerous conidia are being formed. These arise, one at a time, and separately—that is, not in chains—from the apical end of short conidiophores (fig. 4). The conidiophores are usually three or four cells long, and seem identical in the shape and size of their cells with ordinary vegetative mycelium. They were not ob-

served to be club-shaped as described by Appel and Laibach (*1*), but this may be due to differences in the character of the medium used. A gelatinous exudate seemed to accompany the production of spores, which sometimes held numbers of spores together in a perfect sphere, probably due to surface tension, at the end of the condidiophore (fig. 3, A). After a sufficient number of spores had been produced, this tension evidently lessened, allowing the spores to scatter. The entire period from the unripe spore to the mature stage again producing spores was only 40 hours.

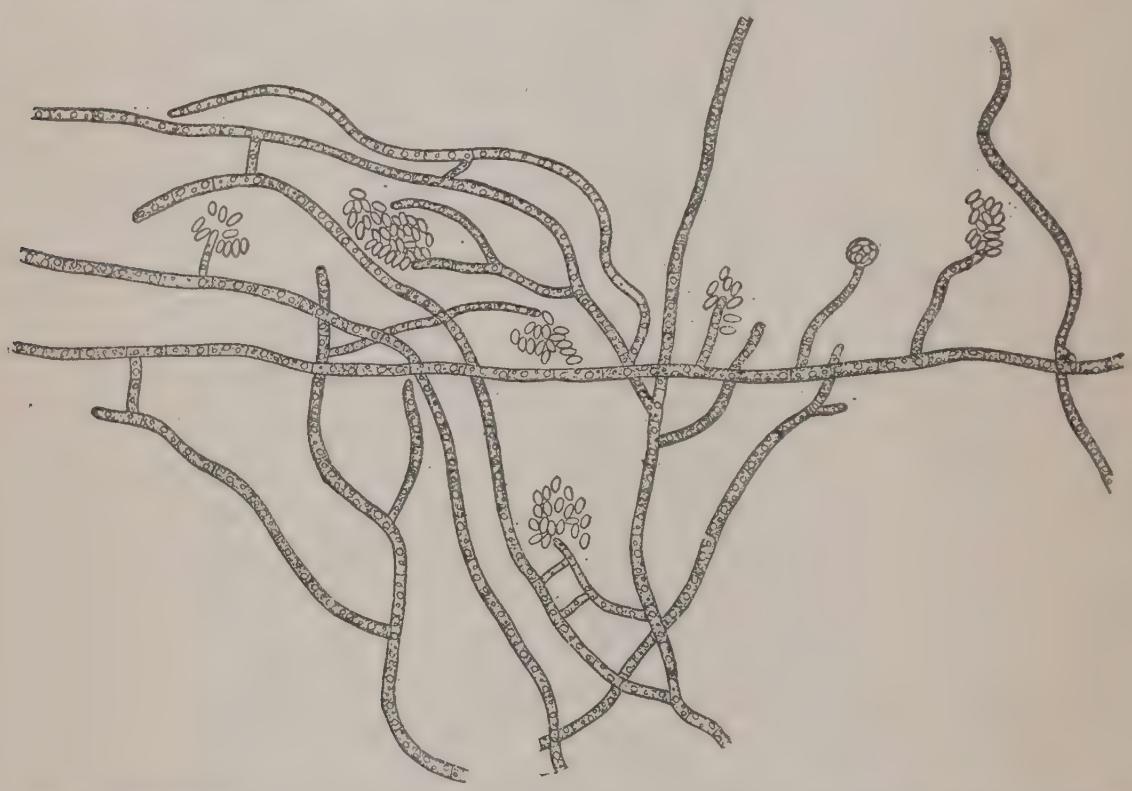

FIG. 4.—*Marssonina panattoniana:* Mycelium, condiophores, and conidia produced on prune-juice agar, 40 hours after the spores were sown (×250).

GROWTH ON VARIOUS MEDIA

Pure cultures of the fungus were transferred to a number of different kinds of media in test tubes to study its reaction to various diets. The use of so many different kinds of media seems now to have been useless labor, as very little difference was observed in the character of growth, except on widely different media. The cultures were all started on the same day, and the results recorded on the fifth day following (Table I).

TABLE I.—*Growth of Marssonina panattoniana in test tubes on various media*

Medium.	Height of growth.	Density.	Rate of growth.	Spore.	Chlamydospore.	Color.
	Mm.					
Prune agar, +30°	1.5	Thick	Rapid	Many	Produced	White.
Pear agar	.1	Sparse	do	do	Not produced.	Do.
Nutrient agar	.5	Medium	Medium.	Few	do	Yellowish white.
Parsnip plug	3–4	Very dense	do	Many	do	White.
Carrot plug	1	Thick	Rapid	Few	do	Do.
Potato plug	3–4	Very dense	do	Many	do	Do.
Glucose agar	2.0	do	Slow	Few	do	Do.
Prune agar, +13°	1.0	Thick	Medium.	Many	do	Do.
Lettuce agar		Dense	Rapid	do	Produced	Hyalin.
Nutrient agar	.5	do	Slow	Few	Not produced.	White.
Corn-meal agar	.5	do	Medium.	Many	do	Do.
Corn meal	1.0	do	Rapid	Few	do	Pinkish white.
Oatmeal	1.0	do	do	do	do	White.

RELATION TO EXTERNAL CONDITIONS

RELATION TO DESICCATION

A drop of spore suspension, prepared by filtering a fresh culture through a sterile funnel containing cotton and glass wool, was placed on each of a number of sterile cover glasses and allowed to dry. The cover glasses were then stored in sterile moist chambers. They were removed one at a time at regular intervals, a drop of sterile water was placed on the dried spores, and the cover slip was inverted over a Van Tieghem ring. The spores were found to withstand desiccation for only four or five days (Table II).

TABLE II.—*Effect of desiccation on the viability of spores of Marssonina panattoniana.*

Trial.	Hour.												Day.						
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7
First	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	o	o
Second	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	o
Third	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	o	o	o

+=Germinated; o=did not germinate.

RELATION OF GERMINATION TO HEAT

The relation of spores to heat was determined by placing Van Tieghem cells containing hanging-drops of spore suspension in the compartments of a differential thermostat whose various compartments maintained even temperatures ranging through about 50 degrees. The thermostat consisted of a long galvanized-iron box 5 by 5 by 30 inches, divided transversely into compartments about 2 inches long. At either end was a somewhat larger compartment, one for ice and the other for water heated to 70° C. by means of a carbon-filament incandescent lamp. The box was insulated on the four sides and bottom with ground cork, and on the top with glass. The slides were hung in the center of each com-

partment on wire hammocks. Each compartment was provided with a short thermometer which could be read through the glass top. The temperatures and percentage of germination are recorded in Table III.

TABLE III.—*Relation of heat to the germination of spores of Marssonina panattoniana: Test with hanging-drop cultures in a differential thermostat*

Time.	Temperature in compartments.								
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.
First trial:	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
12 noon	60	49.5	37	34	28	22.5	17	11.5	6
5 p. m	60	45	35	32	24.5	20	15.5	11	6
6 p. m	60	44	33.5	29	24.5	20	15.5	11	6
8 p. m	60	43	33	28	24	19.5	15	11	6
Percentage of germination	0	0	0	0	66.66	50	15	10	5
Second trial:									
12 noon	58	43	37	32	27	21.5	16	11	5.5
2 p. m	58	44	37.5	31	24	20	15	10.5	5.5
8 p. m	58	44	37	30	24	20	15	10.5	5.5
Percentage of germination	0	0	0	0	85	54	13	30	5

It is readily seen that the fungus does not develop in even moderately high temperatures. This is consistent with the behavior of the fungus in the field and in other laboratory experiments.

THERMAL DEATH POINT OF SPORES

A spore suspension was prepared from a fresh culture and drawn up into capillary glass tubes which were sealed at both ends by flaming in a Meeker burner.[1] These were subjected to various constant temperatures in water baths for 10 minutes and then "shot" into test tubes of melted agar by breaking one end and applying a flame to the other end, after which plates were poured. The results are given in Table IV.

TABLE IV.—*Determination of thermal death point of spores of Marssonina panattoniana in capillary tubes*

No.	Temperature.	Number of colonies.	Remarks.	No.	Temperature.	Number of colonies.	Remarks.
	°C.				°C.		
1	35	About 200		16	42	0	
2	35	About 200		17	42	0	
3	35	About 200	Slight contamination.	18	42	0	
4	38	About 150		19	43	0	
5	38	0	Spores probably failed to "shoot" into agar.	20	43	0	
6	38	About 150		21	43	0	
7	39	61		22	44	0	
8	39	64		23	44	0	Contaminated.
9	39	17	Contaminated.	24	44	0	
10	40	0	Thermal death point.	25	45	0	
11	40	0	3 colonies of bacteria.	26	45	0	
12	40	0		27	45	0	
13	41	0		28	50	0	
14	41	0		29	50	0	
15	41	0		30	50	0	

[1] Novy method (LEVIN, Ezra. THE LEAF SPOT DISEASE OF TOMATO. Mich. Agr. Exp. Sta. Tech. Bul. 25, p. 21, 1916).

According to the method of determining the thermal death point by subjecting spore suspensions of the organism in thin-walled capillaries to various temperatures for 10 minutes, it is seen that the spores of this fungus have a thermal death point of 40° C. for that exposure. The experiment preceding this one showed that their germination did not take place at a much lower temperature. The failure of infection experiments when high temperatures were involved is thus readily explained.

RELATION OF OXYGEN TO GROWTH

A suspension of spores of *Marssonina panattoniana* was prepared from a freshly transferred culture by filtering the entire contents of the test tube through a sterile glass funnel containing glass wool and cotton. A microscopic examination showed that masses of mycelium were separated from the spores by this process. These were then introduced into a test tube of sterile prune-juice agar (+3, Fuller's scale), liquefied at 25° C., and thoroughly shaken. The test tube was then laid away with a slanting surface to cool. After two days the spores at the surface had germinated and made a luxuriant growth, but those in the interior of the medium had ceased to grow immediately after germination. It may be concluded from this that considerable free oxygen is necessary for the development of the fungus.

RECOMMENDATIONS FOR CONTROL

The experiments reported in this paper have shown the usual source of infection of the anthracnose of lettuce, the type of dissemination of the organism, and the relation of the fungus to certain environmental factors. A rational system of control can be based upon these findings.

The anthracnose in the greenhouse or the field commonly starts from the trash of a preceding diseased crop. In the field, rotation is possible, and this, if coupled with the avoidance of manure containing lettuce refuse, will eliminate two important sources of the disease. If further work should show that the fungus is carried with the seed, then some simple system of seed disinfection can probably be readily applied. In the greenhouse, rotation is for the most part impractical. Here careful sanitary measures must prevail. The common practice in greenhouses is to turn under the refuse from a previous crop and to plant immediately in the soil. Naturally with this type of culture there is abundant chance for the infection of the growing crop. Careful collection and destruction of lettuce refuse is recommended. The fertilizer value of the leaves turned under is negligible.

The grower should inspect his beds throughout the growing season, and any plant showing anthracnose, drop, or other severe diseases should be promptly removed from the bed. One plant with the anthracnose can become a center of infection which may involve a whole bed.

Such diseased plants should not be thrown on the manure or compost heap. No doubt the best method of disposing of them is to burn them.

It can not be said that this collection of diseased material can be relied upon to do more than remove the gross sources of infection. It is, furthermore, a preventive measure which is extremely effective against the other lettuce diseases, notably, lettuce-drop and lettuce-graymold.

This disease is commonly found where the splashing or the dripping of water occurs. The repair of gutters or of valves is of obvious necessity. Few growers realize fully the extent of damage which comes from this source. It is not uncommon to see a 10 per cent loss due to the dripping of water the full length of a house. The sprinkling of plants with the hose is an inefficient way of watering. It is rarely necessary to rinse off the leaves of plants in beds, and when hose watering is necessary, the water can be safely and quickly applied by flowing the stream along the ground. Where soil conditions permit, subirrigation is an excellent method.

The most popular method of greenhouse watering is by some type of overhead system. It might seem at first glance that this method would have the same disadvantage as sprinkling with a hose. It has been found by observation that in the overhead systems the splashing from plant to plant, or even from leaf to leaf, does not occur. The jets of water are too fine and the force too slight when the water reaches the plant to bring about any splashing. The writer has no knowledge of an outbreak of lettuce anthracnose in greenhouses watered by the overhead system. The only diseased plants found with such watering systems have always been associated with leaky valves or leaky gutters. The glutinous nature of the spores of the anthracnose fungus explains why these bodies can not be transferred from plants except when the diseased spot is soaked with water. In the field, cultivation should be avoided when the plants are wet. The transference of the disease in the greenhouse is almost wholly brought about by spreading of water. The experiment reported and the diagrams (fig. 2) show most conclusively the relation of this factor.

The recommendation, therefore, is to do away with splashing of water from plant to plant or leaf to leaf. The grower may accomplish this in several ways. The overhead systems are efficient, and are undoubtedly the most popular method of greenhouse watering.

There is possible also the adjustment of the growing conditions so as to favor the lettuce plant and to check the fungus. The most efficient agent in this regard is ventilation. The spores of the fungus will probably not germinate and enter into the lettuce leaf when transferred, unless the leaves stay wet for a considerable time—6 to 12 hours. Since ventilation can not wholly compensate for heavy watering, and as humid conditions will permit germination and penetration of the leaves, excessive watering should be avoided. The most successful lettuce growers aim to keep the

plants, as they say, "on the dry side." This measure is of general benefit with other diseases as well.

High temperature will also check the anthracnose. Lettuce, however, will not make its best growth under such conditions. Hence, the utilization of this factor to prevent the occurrence of the disease is probably of but slight benefit. No doubt in cases of epidemics the disease could be effectively checked by raising the temperature to 30° C. (80° or 90° F.), especially if this were coupled with good ventilation.

So far, the measures outlined have been preventive. These are cheap and readily applied and in line with the best commercial practice. There is left a direct method of fighting this disease. It is possible to protect leaves of lettuce from this disease by spraying them with a protective coat such as Bordeaux mixture or ammoniacal copper carbonate. The latter, while not so effective as fungicide, avoids the staining which accompanies the use of Bordeaux mixture. This method is to be used as a last resort and should not be necessary if the other preventive measures are followed.

SUMMARY

This paper gives the results of experiments with the anthracnose of lettuce and its causal organism.

The signs of the disease consist, in general, of distinct lesions on the leaf blade and midrib. This is frequently accompanied by dwarfing and wilting. Previous workers had found *Marssonina panattoniana* (*M. perforans*) associated with this disease and assumed its etiological relation. This relation has been proved.

A study of the conditions of infection showed that germ tubes were produced from spores in the average time of six hours. Penetration takes place at a point of contact of the walls of the epidermis. Temperatures above 32° C. prevent germination and infection, but excessively wet conditions are not necessary for infection.

Germination phenomena and the relation to various nutrient media were determined. The spores withstand desiccation on glass only four or five days. The fungus does not grow at temperatures above 30° C. The thermal death point was found to be 40° for 10 minutes. The organism does not grow submerged in agar.

The source of the organism in new locations is as yet unsolved. The trash from a previously diseased crop is undoubtedly the chief agent in carrying the disease over from year to year. Splashing, as from a hose in watering, proved to be a ready means of spreading the disease.

Control measures, chiefly prophylactic, based on the findings of this paper are given.

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PLATE C

Leaf of lettuce with lesions of *Marssonina panattoniana*.

PLATE 20

Marssonina panattoniana:

A.—Lettuce leaves of Grand Rapids Forcing variety, showing lesions on midrib and blade.

B.—Lesions on midrib; slightly enlarged.

C.—Lesions on leaves of Black-Seeded Tennis Ball variety produced by artificial inoculation.

A
B
C

THE CALCIUM ARSENATES

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INTRODUCTION

Investigations during the past years on the preparation of the calcium arsenates, together with a study of their chemical and physical properties, indicate the possibility of an economic substitute for the arsenates of lead as an insecticide. During the past two decades the value of the lead arsenates as a stomachic insecticide has been demonstrated. Recently, owing perhaps to conditions brought on by the world war, the cost of this necessary spray material has advanced in price to such an extent that there is a possibility of the curtailing of its use, to the detriment of our orchards and the quality of crops produced.

Heretofore numerous field experiments have been made throughout various sections of the United States to ascertain the practicability of using calcium arsenate as a spray. In most cases these trial experiments have failed, owing to excessive burning of the foliage. As with most arsenicals, the cause of the burn is due to the action of arsenic upon the foliage. The high water-soluble content of commercial samples of calcium arsenate indicates the possibility that therein lies the cause of the intensive burning of foliage. This difficulty was encountered when the commercial lead arsenates were first used, but a study of the properties of laboratory-prepared salts enabled manufacturers to produce a high-grade insoluble lead arsenate that gave orchardists no trouble and caused very little burning. Consequently, it was thought that a more complete knowledge of the composition of the calcium arsenates and a study of the methods of preparation and physical and chemical properties would give an insight relative to the practicability of their use as a substitute for the arsenates of lead.

EXPERIMENTAL WORK

A review of the literature relative to compounds of calcium and arsenic shows that numerous preparations are discussed from a theoretical standpoint, but few have actually been obtained in the laboratory. The calcium arsenates that are of vital interest to us from an insecticidal standpoint must necessarily have such physical and chemical properties that indicate a fairly stable salt. Preliminary experiments convinced us that two salts, the tricalcium arsenate [$Ca_3(AsO_4)_2$] and the calcium hydrogen arsenate ($CaHAsO_4$) appeared to be the only favorable ones.

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Recent work on these two salts is very limited. In 1844 Rammelsberg[1] prepared the pure salt corresponding to the theoretical composition $CaHAsO_4.2H_2O$, containing two molecules of water of crystallization which was further substantiated at about the same date by the work of Klaproth[1] and Dufet.[1] Others, at even an earlier date, obtained different results, indicating variation in amounts of water of crystallization and constitution. Although citations are made to possible methods of preparing the tricalcium arsenate, no data containing actual figures of analysis were found. Furthermore, numerous analyses of commercial samples, both the so-called chemically pure salts, intended for reagents in chemical work, and those sold by manufacturers for spraying purposes proved to be mixtures of various calcium salts, and no two were alike in composition. This point will be further commented upon later.

Previous investigators suggest the preparation of calcium hydrogen arsenate by using calcium chlorid ($CaCl_2$) and sodium hydrogen arsenate (Na_2HAsO_4), the reaction being in accordance with the following equation:

$$CaCl_2 + Na_2HAsO_4 \rightarrow CaHAsO_4 + 2NaCl$$

These salts may, however, also react as represented in the following equation, giving the tricalcium arsenate:

$$3CaCl_2 + 2Na_2HAsO_4 \rightarrow Ca_3(AsO_4)_2 + 4NaCl + 2HCl$$

It is obvious, therefore, that both the arsenates may be formed in greater or less amounts when prepared in the above manner. This may account for the variable composition of the commercial arsenates noted above, and emphasizes more strongly the necessity of specific control of conditions in such a manner that one or the other salt will be produced.

PREPARATION OF CALCIUM HYDROGEN ARSENATE AND TRICALCIUM ARSENATE

In order to make a complete study of those physical and chemical properties of the calcium arsenates that would have an immediate bearing upon their value as a spray material, the preparation of these salts in very pure form is essential.

Numerous methods of preparation were undertaken, but in most cases mixtures were obtained of varying composition. After trial experiments the following method was found to be satisfactory:

Solutions of calcium chlorid and sodium hydrogen arsenate were made, and very slightly acidified with either acetic, hydrochloric, or nitric acid. After filtration, to obtain absolutely clear solutions, the cold calcium-chlorid solution was gradually poured into the sodium-

[1] GMELIN, Leopold, and KRAUT, K. J. HANDBUCH DER ANORGANISCHEN CHEMIE. Aufl. 7, Bd. 3, Abt. 2, p. 569. Heidelberg, 1908. Cites Rammelsberg, Klaproth, and Dufet.

hydrogen-arsenate solution, the solution being constantly stirred. A heavy voluminous precipitate formed immediately and slowly settled; the clear supernatant liquid was decanted off. It was further washed by decantation several times and finally was brought upon a Buchner filter and washed with hot distilled water until free of chlorids. The pure white amorphous powder was then dried at 100° C.

The combined washings were evaporated down to a small volume, when crystals of calcium hydrogen arsenate separated out. After removing the mother liquor by suction, the crystals were washed several times with small quantities of boiling water and dried at 100°.

Analyses made of the samples thus obtained are as follows:

	Total percentage.
Powder:	
Calcium as CaO	28.14
Arsenic as arsenic pentoxid	57.91
Crystals:	
Calcium as CaO	28.40
Arsenic as As_2O_5	58.15
Theoretical composition of $CaHAsO_4.H_2O$:	
Calcium as CaO	28.24
Arsenic as As_2O_5	58.11

Comparing the results with the theoretical composition of $CaHAsO_4.H_2O$ as noted above, we conclude that both samples are pure calcium hydrogen arsenate, containing one molecule of water of crystallization.

When these samples were further dried at 175° C., both lost their water of crystallization, as shown by the following analytical results:

	Total percentage.
Powder:	
Calcium as CaO	31.10
Arsenic as As_2O_5	63.80
Crystals:	
Calcium as CaO	31.20
Arsenic as As_2O_5	63.83
Theoretical composition of $CaHAsO_4$:	
Calcium as CaO	31.13
Arsenic as As_2O_5	63.86

A comparison of these results with the theoretical composition of anhydrous calcium hydrogen arsenate shows each of the samples to be a pure calcium hydrogen arsenate.

When the heating is continued at 230°, the tendency to form the pyroarsenate ($Ca_2As_2O_7$) is observed as shown by the analysis:

	Total percentage.
Calcium as CaO	32.01
Arsenic as As_2O_5	65.78

Another method found successful in preparing pure calcium hydrogen arsenate consists in dissolving a commercial calcium arsenate, which may be a mixture of the two calcium arsenates under discussion, in the smallest quantity possible of either hydrochloric or acetic acid, as large a volume of water as convenient being maintained. When evaporated upon a hot plate, either amorphous powder or crystals separate out, depending upon rapidity of evaporation.

A simple laboratory method that also proved successful consists in adding slowly a solution of calcium hydroxid [$Ca(OH)_2$] to a clear aqueous solution of arsenic acid, and in stirring vigorously meanwhile, until the acid is about three-fourths neutralized. By slow evaporation crystals of pure calcium hydrogen arsenate separate out.

The chief precaution that must be observed in order to obtain the pure salt is to maintain an excess of acid or H-ion in the original solutions. This excess of acid, however, must be at a minimum, or the precipitate will redissolve.

In the preparation of tricalcium arsenate three methods suggest themselves from a theoretical standpoint, as indicated by the following equations:

(1) When pure calcium hydrogen arsenate is acted upon by an alkali:

$$3CaHAsO_4 + 2NaOH \rightarrow Ca_3(AsO_4)_2 + Na_2HAsO_4 + 2H_2O$$

(2) When arsenic acid is completely neutralized with a solution of calcium hydroxid:

$$2H_3AsO_4 + 3Ca(OH)_2 \rightarrow Ca_3(AsO_4)_2 + 6H_2O$$

(3) When solutions of calcium chlorid and sodium arsenate are allowed to react:

$$3CaCl_2 + 2Na_3AsO_4 \rightarrow Ca_3(AsO_4)_2 + 6NaCl$$

In all cases cited, if the reaction is permitted to reach an equilibrium and conditions are controlled as indicated, it is possible pure tricalcium arsenate would result. However, the last proved to be the most practical, and concordant results were obtained. The perfected method is as follows: Cold solutions of both calcium chlorid and sodium hydrogen arsenate were prepared and the former made just sufficiently alkaline that no calcium hydroxid precipitated out. To the sodium-hydrogen-arsenate solution just enough sodium hydroxid was added to change completely the salt to sodium arsenate. (Special care must be taken lest too much sodium hydroxid should be added, as this would form calcium hydroxid when combined with the calcium-chlorid solution.) Both solutions were then filtered and the clear calcium-chlorid solution was added to the sodium-arsenate solution. A heavy voluminous precipitate formed that settled very slowly. This was washed by repeated centrifuging and decanting off the supernatant liquid each

time. It was finally brought upon a Buchner filter and washed free of chlorids with hot water. After drying in an oven at 100°, the analysis gave the following results:

	Total percentage.
Calcium as CaO	38.49
Arsenic as As_2O_5	52.50

These results indicate that a salt is produced containing two molecules of water of crystallization and agreeing with the formula $Ca_3(AsO_4)_2 . 2H_2O$

When further dried at 175°, the pure calcium arsenate was obtained as shown by the following analytical data:

	Total percentage.
By analysis:	
Calcium as CaO	42.16
Arsenic as As_2O_5	57.73
Theoretical composition of $Ca_3(AsO_4)_2$:	
Calcium as CaO	42.20
Arsenic as As_2O_5	57.80

SPECIFIC GRAVITY, SOLUBILITY, AND RELATIVE STABILITY OF CALCIUM HYDROGEN ARSENATE AND TRICALCIUM ARSENATE

A spray material in powder form must be in a fine state of subdivision to facilitate efficient spreading on foliage and must have a specific gravity sufficiently low that it will remain in suspension in water for a considerable length of time.

The specific gravity was obtained by determining the weight of absolute alcohol displaced by a known quantity of the salt. The alcohol was specially prepared by redistilling the commercial chemically pure absolute alcohol over calcium oxid obtained by igniting chemically pure calcium carbonate ($CaCO_3$). All weighings were made at 20° C. with recently standardized weights. The specific gravity for the different salts was found to be as follows:

Calcium hydrogen arsenate ($CaHAsO_4 . H_2O$) at 20°/4°	3.09
Anhydrous calcium hydrogen arsenate ($CaHAsO_4$) at 20°/4°	3.48
Tricalcium arsenate [$Ca_3(AsO_4)_2 . H_2O$] at 20°/4°	3.23
Anhydrous tricalcium arsenate [$Ca_3(AsO_4)_2$] at 20°/4°	3.31

The solubility of an arsenical utilized for insecticidal purposes is an exceedingly important factor. The presence of an excess of soluble arsenic or any salt that would ultimately pass into solution, forming arsenic acid or a soluble arsenate, would cause burning of foliage and render the arsenical useless for spraying purposes. Qualitative tests showed that both calcium hydrogen arsenate and tricalcium arsenate were slightly soluble, and, like many other calcium salts, the solubility is greater at lower than at higher temperatures. The determinations were made at 25° C. in a constant-temperature bath that was fitted with a revolving bottle holder. By means of the latter, four bottles could be submerged and kept in a state of constant agitation during the

entire experiment. Various amounts of the samples were introduced into the different bottles, and 300 c. c. of conductivity water were added to each. They were then placed in the constant-temperature bath and allowed to revolve for five days, when 100 c. c. were removed from each bottle and evaporated on a steam bath. The bottles were replaced in the bath and allowed to continue three days longer, when another 100 c. c. was removed and evaporated as before.

Finally, after evaporation the salts were dried in an electric oven at 100° C. and then at 175°, as recorded in Table I.

TABLE I.—*Comparative solubility at 25 °C. of calcium hydrogen arsenate and tricalcium arsenate*

Salt.	Drying temperature.	Weight (grams in 100 gms. of water).
	°C.	
Calcium hydrogen arsenate	100	0.3308
Do	175	.3108
Tricalcium arsenate	100	.0140
Do	175	.0133

Analysis of the dissolved material showed that the composition of the latter was the same as that of the original salt used, indicating no perceptible hydrolysis.

We observe from the results given in Table I that at 25° C. the calcium hydrogen arsenate is far more soluble than the tricalcium arsenate. This point is of considerable practical importance, since the solubility of the calcium hydrogen arsenate proximates the amount shown by field experiments to cause burning of foliage. These data indicate the futility of attempting to use the pure calcium hydrogen arsenate alone as a spray. The tricalcium arsenate, on the other hand, is only slightly soluble, and the danger of burning from that source would probably be negligible.

From the standpoint of density and solubility, it is probable that the tricalcium arsenate could be safely used as a spray material, while the calcium hydrogen arsenate is doubtful. An important factor remains yet to be taken into consideration, namely, the stability of the salts. This was ascertained by a study of the chemical change that resulted when either the calcium hydrogen arsenate or tricalcium arsenate was shaken at intervals for several days in solutions of acids, both organic and inorganic, ammonium hydroxid, sodium hydroxid, sodium chlorid, and similar salts. In all cases tried the calcium hydrogen arsenate reacted, dissolved, or arsenic in soluble form was found in solution. However, the tricalcium arsenate manifested greater stability and in many instances showed only slight reactivity. Inferring from the relative stability of the two salts, both arsenates would under severe and abnormal climatic conditions probably yield to the action of carbon dioxid and moisture in

the air with the consequent formation of free arsenic acid. The tricalcium arsenate being more insoluble and more stable than the calcium hydrogen arsenate would not have the tendency to react so readily and the danger attending its use would be diminished accordingly.

EFFECT OF CALCIUM HYDROXID ON SOLUBILITY OF THE CALCIUM ARSENATES

Our investigations thus far indicate favorable possibilities for the use of the calcium arsenates. The presence of any substance that would prevent solubility and reactivity would prove beneficial in the use of tricalcium arsenate and almost a necessity in the case of the calcium hydrogen arsenate. To judge from a theoretical standpoint, ordinary quicklime (CaO) would fulfill these requirements. The calcium hydroxid, on becoming soluble, would react with any arsenic that goes into solution, forming more calcium arsenate.

In order to corroborate the above assumption, different amounts of the pure salts were introduced into each of several 200-c. c. graduated flasks together with known quantities of lime (CaO). In some cases calcium carbonate ($CaCO_3$) was also added. The flasks were then made up to mark with distilled water and shaken at intervals for two days. After allowing the salts to settle, determinations were made for total arsenic and calcium in the clear supernatant liquid. The results are given in Table II.

TABLE II.—*Effect of calcium hydroxid on the solubility of the calcium arsenates. Time, two days*

Flask No.	Substances used.	Quantity added.	Quantity of arsenic pentoxid found.	Quantity of calcium oxid found.
		Gm.	*Gm.*	*Gm.*
I	Tricalcium arsenate	1.0	0.0084	0.0049
	Calcium oxid	.2		
	Calcium carbonate	.8		
II	Tricalcium arsenate	1.5	None.	.1131
	Calcium oxid	.7		
	Calcium carbonate	None.		
III	Calcium hydrogen arsenate	1.0	None.	.0554
	Calcium oxid	.5		
	Calcium carbonate	None.		
IV	Calcium hydrogen arsenate	1.0	.0026	.0082
	Calcium oxid	.2		
	Calcium carbonate	.8		
V	Calcium arsenate, C. P. (J. T. Baker)	2.0	None.	.0573
	Calcium oxid	1.0		

Inspection of the results given in Table II indicates that wherever calcium oxid is present in even slight excess, so that calcium hydroxid was found qualitatively in solution, no soluble arsenic was detected.

Furthermore, the calcium carbonate prevented in no way the solubility of the calcium arsenates. It is quite evident, therefore, that if there is an excess of calcium hydroxid in the system, the solubility of both calcium arsenates is inhibited.

In order to substantiate the former results and to ascertain whether or not, by allowing the action to continue for a longer time, the system would come to an equilibrium and continue indefinitely with no arsenic in the solution, a similar set of flasks was prepared. These were allowed to stand for two weeks, with an occasional shaking, and then the supernatent liquid was analyzed as before. The quantity of material used, together with the results obtained, is given in Table III.

TABLE III.—*Effect of calcium hydroxid on the solubility of the calcium arsenates. Time, two weeks*

Flask No.	Substances used.	Quantity added.	Quantity of arsenic pentoxid found.	Quantity of calcium oxid found.
		Gm.	*Gm.*	*Gm.*
I	Tricalcium arsenate	1.0	None.	0.2481
	Calcium oxid	.5		
II	Calcium hydrogen arsenate	1.0	None.	.2451
	Calcium oxid	.5		
III	Calcium hydrogen arsenate	1.0	None.	.2540
	Calcium oxid	1.0		

The results given in Table III further verifies those in the previous experiment and emphasizes the fact that a definite point of equilibrium is reached in which the concentration of calcium hydroxid in solution becomes constant. Furthermore, it was found that the concentration of calcium hydroxid is the same as the maximum solubility of calcium hydroxid in pure water at the same temperature.

From a practical standpoint the preventive action of calcium hydroxid on the solubility of the calcium arsenates is of vital importance. The free calcium hydroxid has no harmful effects upon foliage. If, therefore, 1 part of quicklime is added for every 2 parts of either calcium hydrogen arsenate or tricalcium arsenate as a combination spray, no burning of foliage should result from the solubility of the arsenates. Furthermore, the action under severe atmospheric conditions will be greatly diminished, since the carbon dioxid in the air will first react with the calcium hydroxid, forming the harmless calcium carbonate before the calcium arsenates are effected.

ACTION OF CARBONIC ACID UPON THE CALCIUM ARSENATES

A study of the action of carbon dioxid upon the calcium arsenates is an important consideration. Although chemical changes due to carbonic acid, or a saturated water solution of carbon dioxid, as noted in

laboratory experiments, would be more vigorous than the action of the carbon dioxid of the atmosphere, a similar but much slower change would probably occur. In order to ascertain whether any reaction may obtain, a series of flasks, each of which contained a specific quantity of calcium arsenate and 50 c. c. of distilled water was prepared. Carbon dioxid was then passed through the mixture, keeping the salts in a constant state of agitation. After 10 hours the supernatant liquid was boiled to expel excess carbon dioxid and a determination was made for arsenic in solution. The results, together with amounts of arsenate used, are given in Table IV.

TABLE IV.—*Effect of carbolic acid on the solubility of the arsenates*

Salt used.	Quantity taken.	Quantity of arsenic as arsenic pentoxid in 50 c. c.
	Gm.	*Gm.*
Tricalcium arsenate	2	0.0208
Calcium hydrogen arsenate	2	.0428
Tricalcium arsenate+0.5 gm. of calcium oxid	1	.0012
Calcium hydrogen arsenate+0.5 gm. of calcium oxid	1	.0046
Calcium hydrogen arsenate+1 gm. of calcium oxid	1	.0020

From the above results it is plainly evident that carbonic acid has a solvent action upon the calcium arsenate. Furthermore, when the excess carbon dioxid was boiled off, a precipitate, consisting of a mixture of calcium carbonate and a smaller amount of calcium arsenate, was thrown down. The presence of calcium hydroxid, however, diminished the solvent action appreciably.

REACTION BETWEEN THE CALCIUM ARSENATES AND LIME-SULPHUR SOLUTION

Combination sprays are of great economic importance, since time, labor, and money are thereby saved. The tendency for chemical reaction that destroys or greatly diminishes the efficiency of both spray materials often results, and consequently prevents their use in this manner. The combination of lead hydrogen arsenate and lime-sulphur exemplifies this difficulty, as shown by Robinson and Tartar.[1] The concentration of the lime-sulphur is reduced; arsenic is found in solution; lead sulphid is precipitated out, thus utilizing part of the lead arsenate; and severe burning of foliage may occur, depending upon weather conditions.

When pure tricalcium arsenate was added to dilute lime-sulphur, no reaction appeared to occur. Sanders[2] in field experiments reports

[1] ROBINSON, R. H., and TARTAR, H. V. THE ARSENATES OF LEAD. Oreg. Agr. Exp. Sta. Bul. 128, 32 p., 2 fig. 1915.

[2] SANDERS, G. E. THE EFFECT OF CERTAIN COMBINATIONS OF SPRAYING MATERIALS ON THE SET OF APPLES. *In* Proc. Ent. Soc. Nova Scotia, 1916, p. 17–21. 1917.

more favorable results obtained with the calcium-arsenate-lime-sulphur combination spray than with lead-arsenate-lime-sulphur. Hence, a study of the chemical reaction, if any, was initiated. Pure lime-sulphur was prepared in the laboratory from crystallized sulphur and calcium oxid, the latter being obtained by igniting calcium carbonate. The lime-sulphur produced had a specific gravity of 1.242, or 28.2° Baumé, and was diluted to average field spraying strength. Both of the calcium arsenates were prepared in the laboratory in pure form. Into each of several flasks 1 gm. of tricalcium arsenate, 1 gm. of calcium hydrogen arsenate, or 1 gm. of calcium hydrogen arsenate + 0.5 gm. of calcium oxid was introduced, and 200 c. c. of the diluted lime-sulphur were added. A control flask containing 200 c. c. of the diluted lime-sulphur only was also prepared. The flasks were then shaken at intervals during two days, after which the salts were allowed to settle and determinations were made for total sulphur, calcium oxid, and arsenic pentoxid in the supernatant lime-sulphur solution. Table V records the results, expressed in grams contained in 200 c. c.

TABLE V.—*Reaction between lime-sulphur and the calcium arsenates*

Constituent.	Control.	Lime-sulphur and tricalcium arsenate.	Lime-sulphur and calcium hydrogen arsenate.	Lime-sulphur and calcium hydrogen arsenate + calcium oxid.
	Gm.	*Gm.*	*Gm.*	*Gm.*
Sulphur ..... total..	1.8400	1.8450	1.8490	1.8420
Calcium oxid ..... total..	.8810	.8840	.8820	1.1090
Arsenic pentoxid ..... total..	.....	None.	None.	None.

The above results proved favorable beyond expectation. There appears to be no chemical change whatsoever in the lime-sulphur solution, all constituents remaining constant and agreeing with results given for the control solution. Barring the possibility of chemical reaction after spraying, we have in the calcium arsenate an ideal spray material to be used in combination with lime-sulphur. The efficiency of the latter is not reduced and the calcium arsenate remains unchanged.

Attention is further brought to the analysis of the liquid in the flask containing calcium hydrogen arsenate + calcium oxid. As with calcium hydrogen arsenate and tricalcium arsenate, no arsenic was found in the lime-sulphur solution, and the sulphur content remained constant. The amount of calcium oxid, however, exceeds that obtained in the other flasks. This increase of the calcium oxid is equivalent to the solubility of calcium hydroxid in pure water at the temperature under observation. Since the addition of lime in no way reacts with either of the other spray

materials, it might be advisable to have it present in order to prevent danger of a foliage burn due to severe atmospheric conditions. This would be especially true of the less stable calcium hydrogen arsenate. This precaution, however, must be verified by field experiments.

Since the above conclusions were obtained on pure samples, the experiment was repeated with commercial products in order to ascertain the practicability of their use. A solution of commercial lime-sulphur having a specific gravity of 1.227, or 26.7° Baumé, was diluted in the same proportion as indicated in the previous experiment. A commercial calcium arsenate was used that had the following composition:

Calcium oxid	total	39.20 per cent.
Arsenic pentoxid	total	48.90 per cent.
Calcium carbonate	total	1.14 per cent.

From this analysis we infer that the sample consisted of over 60 per cent of tricalcium arsenate and the remainder calcium hydrogen arsenate and calcium carbonate.

Treatment was similar to that of the last experiment, and the composition of the lime-sulphur solution is given in Table VI.

TABLE VI.—*Reaction between commercial calcium arsenate and lime-sulphur*

Substance used.	Calcium oxid.	Sulphur.	Arsenic pent-oxid.
	Gm.	*Gm.*	*Gm.*
Control (lime-sulphur only)	0.7821	1.6482	.....
Calcium arsenate and lime-sulphur	.7816	1.6474	None.

Here again, we observe that no apparent chemical action has occurred. No arsenic was found in solution, and the total calcium oxid and sulphur content remained the same. It is obvious that a commercial sample composed of both calcium hydrogen arsenate and tricalcium arsenate and possibly other impurities may be combined with commercial lime-sulphur with the same degree of safety as the pure laboratory products.

The use of dry substitutes for lime-sulphur is rapidly becoming prevalent; hence, a study of their combination with calcium arsenate would be beneficial. Consequently 0.5 gm. of a commercial "dry lime-sulphur" and the same quantity of a so-called "soluble sulphur" which is composed chiefly of the sulphids of sodium or potassium were used. Each was introduced into 200-c. c. graduated flasks, together with 1 gm. of commercial calcium arsenate, and each mixture was then made up to mark with water. After shaking occasionally during two days the mixture in both flasks was allowed to settle and was analyzed for arsenic in solution, as reported in Table VII.

TABLE VII.—*Solubility of commercial calcium arsenate used with dry lime-sulphur*

Substance used.	Arsenic pentoxid in 200 c.c.
	Gm.
Control	None.
Dry lime-sulphur	None.
Soluble sulphur	0.230

These results indicate that no chemical changes occurred when calcium arsenate and dry lime-sulphur were combined. The soluble-sulphur compound, however, caused such a large quantity of arsenic to pass into solution that would make it inadequate for utilization as a combination spray.

VALUATION OF COMMERCIAL SAMPLES

There are at present on the market various calcium-arsenate products offered as substitutes for lead arsenate, together with the so-called chemically pure salts used as reagents for chemical work. Complete analyses have been made of these samples, both to ascertain whether or not they approximated the theoretical composition of the pure salt and also to estimate their commercial value as spray materials. Table VIII gives a compilation of the results obtained.

TABLE VIII.—*Composition of samples of commercial calcium arsenate*

Sample.	Calcium oxid.	Arsenic pentoxid.	Calcium carbonate.
	Per cent.	*Per cent.*	*Per cent.*
Kahlbaum (C. P.)	36.62	51.50	Trace.
Baker (C. P.)	40.96	42.75	7.25
Commercial No. 1	43.46	40.80	40.00
Commercial No. 2	39.20	48.86	1.14
Commercial No. 3	45.61	19.22	8.40

The wide variation in the composition of commercial arsenates is plainly evident from the results given in Table VIII. Both Kahlbaum's and J. T. Baker's chemically pure samples, supposed to be high-grade salts, are mixtures of the calcium hydrogen arsenate and tricalcium arsenate, with between 10 per cent and 20 per cent water of crystallization or constitution. Furthermore, both the Kahlbaum and Baker samples contained some calcium carbonate. Commercial samples 1, 2, and 3 likewise emphasize the necessity of greater care in their manufacture. Attention is especially called to sample 1. It contains 40 per cent of calcium carbonate, but only 43.46 per cent of total calcium, estimated as calcium oxid. These figures indicate that there is insuffi-

cient base to combine with the quantity of arsenic pentoxid present, and further examination showed the latter was there as actual arsenic pentoxid uncombined. It is probable that the manufacturer attempted to prepare the calcium arsenate from arsenic pentoxid and an impure lime which was chiefly calcium carbonate. The latter would react very slowly, if at all, and the excess arsenic pentoxid over the amount required to combine with the calcium hydroxid would remain unchanged as noted above. If the manufacturer would exercise a more complete control of conditions and know definitely what materials are being used, a spray material sufficiently pure for practical use could probably be obtained.

Thus far our investigation has shown the possibility of the calcium arsenates' being a practical, economical, and evidently satisfactory substitute for the more expensive lead arsenate. The pure salts contain 57.8 and 63.9 per cent of arsenic calculated as arsenic pentoxid for the tricalcium arsenate and calcium hydrogen arsenate, respectively, while the hydrogen and basic lead arsenate contains only 33.1 and 25.5 per cent of arsenic pentoxid, respectively; in other words, the calcium arsenates contain more than twice as much arsenic, the active killing agent of the insecticide. For practical spraying purposes, therefore, only one-half of the quantity of calcium arsenate should be used, compared with lead arsenate. This point must, however, be more definitely determined by field experiments, since there is a bare possibility that the lead has toxic values that the calcium does not have. Relative toxic values and killing efficiency has been recently shown in laboratory experiments with the "common tent caterpillar" by Lovett and Robinson [1] and with the "fall webworm" by Scott and Siegler,[2] indicating equal killing efficiency of calcium arsenate compared with lead arsenate.

Field spraying experiments have been carried on by several investigators with more or less successful results. This may be due to the use of an unreliable calcium arsenate similar to the one cited above. Sanders,[3] however, reports very favorable results during the past two seasons. Likewise, Scott and Siegler [4] obtained encouraging results.[5]

In cooperation with Prof. A. L. Lovett, Entomologist at this Station, preliminary field experiments were tried with the pure salts prepared in the laboratory. Favorable results, especially with tricalcium arsenate plus calcium oxid were obtained, but owing to insufficient time and late-

[1] LOVETT, A. L., and ROBINSON, R. H. TOXIC VALUES AND KILLING EFFICIENCY OF THE ARSENATES. *In* Jour. Agr. Research, v. 10, no. 4, p. 199–207. 1917.

[2] SCOTT, E. W., and SIEGLER, E. H. MISCELLANEOUS INSECTICIDE INVESTIGATIONS. U. S. Dept. Agr. Bul. 278, 47 p. 1915.

[3] SANDERS, G. E. ARSENATE OF LEAD VS. ARSENATE OF LIME. *In* Proc. Ent. Soc. Nova Scotia, 1916, p. 40–45. 1917.

[4] SCOTT, E. W., and SIEGLER, E. H. Op. cit.

[5] Scott and Siegler gave the analysis of the calcium arsenate used as showing only 0.04 per cent of soluble arsenic oxid. A possible explanation is that the spray material was prepared from stone lime (containing 80 per cent of calcium oxid), sodium arsenate, and water. A very slight excess of the stone lime would prevent any arsenic from becoming soluble, as shown in our experiments reported above.

ness in the season, the trials were limited. During the coming spraying season more elaborate field experiments will be conducted by Prof. Lovett and reported upon at a later date.

SUMMARY

(1) This paper reports a chemical study of the calcium arsenates.

(2) Pure calcium hydrogen arsenate ($CaHAsO_4$) and tricalcium arsenate [$Ca_3(AsO_4)_2$] have been prepared and methods for their preparation outlined.

(3) The specific gravity for calcium hydrogen arsenate was found to be 3.48; that for tricalcium arsenate 3.31.

(4) The solubility of calcium hydrogen arsenate in 100 gm. of water at 25° was 0.310 gm. and that of tricalcium arsenate was 0.013 gm.

(5) A chemical study of the relative stability showed that (a) there was no apparent reaction between either calcium hydrogen arsenate or tricalcium arsenate and lime-sulphur when combined at a dilution used in field spraying. (b) The addition of excess of calcium oxid to either of the calcium arsenates prevented arsenic from going into solution. (c) Some commercial substitutes for lime-sulphur reacted with both of the calcium arsenates. (d) The arsenates reacted with or became soluble in organic acids and various salts, such as sodium chlorid.

(6) The composition of various commercial arsenates is given and commented upon.

STEMPHYLIUM LEAFSPOT OF CUCUMBERS[1]

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INTRODUCTION

In the latter part of September, 1915, the writer discovered a peculiar leafspot on cucumbers (*Cucumis sativus*) in a small garden near Plymouth, Indiana. The spots showed a distinctly mottled effect, and in some cases were so numerous as to entirely kill the leaf. Specimens of this same disease were discovered about the same time by Mr. W. W. Gilbert, in two cucumber fields, one at Lapaz and the other at Lakeville, Ind., and later, in a field at Bowling Green, Ohio. A field was found a few miles from Plymouth in which over 40 per cent of the plants were affected by this disease. On many of these plants the leaves were so badly affected as to considerably reduce the yield. During the summer of 1916, no cucumbers were grown in any of the fields above mentioned and although a sharp watch was maintained, the disease was not found in the vicinity of Plymouth. However, while making a survey of the cucumber fields in northern Indiana about the middle of September, 1916, the disease was again found in fields around Hamlet, Lapaz, and North Liberty. While the disease was not sufficiently widespread to prove a serious economic factor in the growing of cucumbers, observations indicate that in those spots of the field in which it occurred the leaves were so badly injured as to reduce appreciably the yield for pickles.

SYMPTOMS OF THE DISEASE

The disease first appears in the form of small yellowish spots on the leaf, usually 0.5 to 1 mm. in diameter. The spots are soon visible on both the upper and lower surfaces of the leaf (Pl. 21, A, B). The

[1] These investigations were carried out at Plymouth and Lafayette, Ind., by the Office of Cotton and Truck Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Purdue University Agricultural Experiment Station.

[2] The writer wishes to express his thanks to Prof. H. S. Jackson, of Purdue Experiment Station, and to Mr. W. W. Gilbert, of the Bureau of Plant Industry, for helpful suggestions and criticisms during the progress of this work.

center of the upper surface of the spots soon changes to a light yellowish brown with a reddish brown border. Around this border for 1 to 2 mm. the leaf becomes light green. In some cases the spots may be almost white. The color depends apparently on the amount of sunlight as well as on the humidity. On the lower surface the spots remain lighter colored and do not develop the dark border. Mature lesions are of two distinct types; one a small spot, the other a large mottled spot, depending on the abundance of infection and on weather conditions. The small spots are produced most abundantly when infection is followed by dry weather. They range from 0.2 mm. to 3 or 4 mm. in diameter, the majority being 1 to 2 mm. (Pl. 21, A, B). At first circular in outline, they may become irregular or markedly angular, being limited by the veins of the leaf. As already mentioned, there may be considerable variation in the color of the spots, in some cases the reddish brown border being almost entirely absent.

When infection is followed by moist weather, and especially if several spores germinate near each other in a large drop of water, a different type of lesion is produced. These lesions are larger, ranging from 4 to 15 mm. in diameter (Pl. 22, A, B). They vary from white to light brown, even in the same spot, with reddish-brown areas along the veins. This gives them a characteristic mottled appearance. These large spots may originate in various ways. In some cases the infection appears over the large spot all at once; in others it first appears as several small lesions which later unite; while in others the infection may later extend outward from a central point. In the last-named case there may be a dark-brown center of 3 to 5 mm. with a lighter area around it. On the lower surface of the leaf the mottled appearance is much less distinct, the spots usually being light yellowish with occasionally a slight tinge of brown. During hard rains the centers of these spots may be broken out, leaving holes in the leaves. In severe cases the leaf may be so badly infected that it turns yellow or brown and dies.

The writer has not seen infection on stems, petioles, or fruit in the field, but occasional longitudinal lesions on stems and petioles have been produced by artificial inoculation in the greenhouse. These spots are slightly sunken, 0.5 to 1 mm. wide by 3 to 5 mm. long, with a very light yellowish-brown center and darker border.

ISOLATION OF THE CAUSAL ORGANISM

Material collected both in 1915 and 1916 was invariably found to have associated with the spots a fungus belonging to the Dematiaceae-Dictyosporae group of the Hyphomycetes.

Since the fungus produces rather large spores on a surface mycelium, spore dilution in plate cultures was found to be the easiest method of obtaining it in pure culture. Young spots free from dirt on which there was abundant spore formation were inverted under a binocular micro-

scope and a few spores transferred by means of a sterile, moistened scalpel to string-bean agar. Dilution plate cultures were then made. As soon as the spores had germinated, as shown by examination under a microscope, single spores were transferred to other agar plates. When these cultures produced spores, single-spore isolations were again made to make doubly sure they were free from contamination. In a few cases the fungus was isolated by the tissue-culture method from leaves. Infected leaves were sterilized by washing for one-half to three minutes in a 1 to 1,000 solution of mercuric chlorid or in 95 per cent alcohol. They were then thoroughly washed with distilled water and plated in string-bean or potato agar. In some cases these cultures were overrun by various mold fungi, but in others a fungus identical in all respects with that obtained from the spore dilutions was isolated. The fungus was isolated from material collected in 1916 at Lapaz and Hamlet, Ind. These isolations were labeled as strains 1 and 2, respectively. A tissue culture isolation from Lapaz was labeled "strain 3." Cultures from two of the mold fungi were retained and used for later inoculations.

INOCULATION EXPERIMENTS

Inoculation tests were made with cultures from the strains mentioned above as well as with spores taken directly from diseased cucumber leaves. All strains, as well as the fresh spores, developed abundant infection. Inoculations made with the other fungi isolated from the diseased spots failed to develop infection. Most of the inoculations were made in the greenhouse, only one series being made in the field. In both cases the inoculations were successful.

One series of inoculations made on September 13, 1916, will be described in detail as being typical of the manner in which all were made. Spores of strains 1, 2, and 3 were taken from string-bean-agar cultures 15 days old. Contemporaneous germination tests on slides showed that the spores were viable. Inoculations were made with suspensions of these spores in distilled water, tap water, and beef bouillon. The spores were applied to both upper and lower surfaces of the leaves by spraying the suspension from an atomizer or by dropping it from a pipette. In spraying with the atomizer some of the spores doubtless occasionally reached both surfaces. Four plants were used with each strain for each different method of inoculation, 12 plants being sprayed with water and 6 with bouillon as controls. One-half of the plants were left in the dry air of the greenhouse. The others were placed for 24 hours in a glass culture chamber so arranged that fresh air was admitted from below, and after this period they were also kept in the greenhouse. The results on September 17, four days later, are summarized in Table I.

TABLE I.—*Infection showing on September 17, 1916, on cucumbers inoculated on September 13*

Manner of inoculation.	Infection on plants kept in—	
	Culture chamber for 24 hours.	Greenhouse from beginning.
Leaves sprayed on upper surface	Fine spotting (0.2 to 0.75 mm. in diameter).	No infection
Leaves sprayed on lower surface	.....do.....	Do.
Leaves with drops on upper surface.	Fine spotting in groups.....	Do.
Leaves with drops on lower surface.	.....do.....	Do.
Controls.....	No infection.....	Do.

Infection occurred equally well with all the strains used and regardless of whether the spores were applied in distilled water, tap water, or beef bouillon.

An inspection of Table I shows the following points: The infection appeared on both surfaces regardless of which surface had been inoculated. Infection appeared only on those plants kept in a moist atmosphere for some hours after inoculation. As might be expected, those leaves that were sprayed with the atomizer showed a fine spotting over more or less of the entire surface, while on those inoculated with large drops of liquid the spots occurred in groups. In this series of inoculations the spots did not continue to enlarge and spores were produced on only a few of the lesions. The fungus was reisolated both from the spots that produced spores and from those that did not.

The question then arose as to the cause of the failure to produce the large mottled spots with normal spore formation so common in the field. The plants had been kept in the greenhouse at a rather high temperature and low humidity. Accordingly another series of inoculations was started in which these factors were varied. It was found that under conditions of fairly high humidity and of moderate temperature the characteristic mottled spots were produced with abundant spore formation on the lower surface of the leaves. Under conditions of excessive humidity and partial shade, spore formation occurred on both surfaces of the leaf, a phenomenon very rare in nature.

Other inoculations, in which the writer used mycelium in comparison with suspensions of spores, have shown that infection occurs equally well in either case.

On September 15, 1916, a series of inoculations was made on cucumber, gourd, and squash (*Cucurbita* spp.) with a suspension of spores in water. The plants were placed in the culture chamber for 24 hours and then kept in the greenhouse. Infection appeared on the cucumbers, but not on the gourds or squashes. Later, these experiments were repeated, the

plants being kept under conditions more favorable for infection. In this case infection occurred on all the inoculated plants, cucumbers, egg and pear-shaped gourds, crookneck and White Bush Scalloped squashes (Pl. 23, A. B). These exepriments have been repeated in the field with similar results.

Repeated attempts have been made to obtain infection on other parts of the host than the leaves. In the case of cucumbers only, an occasional infection on stem and petiole has resulted when the plants were kept continuously in the culture chamber.

The various inoculation experiments have afforded abundant opportunity for observing the effect of age on the susceptibility of leaves to infection. Infection may occur on the youngest unfolding leaves, but is much more abundant on the older leaves. The spots develop more normally on the older leaves, and spore formation occurs earlier and more abundantly.

Through the inoculation experiments outlined above, the parasitism of this fungus to the leaves of cucumbers, and certain varieties of gourds and squashes would seem to be clearly established. Successful inoculations were made on the following four varieties of cucumbers: Arlington White Spine, Nichol's Medium Green, Heinz Muscatine, and Davis Perfect.

TAXONOMY AND DESCRIPTION OF THE FUNGUS

This fungus belongs in the family Dematiaceae of the Hyphomycetes. The dark, muriform, subglobose conidia place it in the group Dictyosporae. Careful consideration of the fungus, both in the field and in artificial culture has led the writer to place it in the genus Stemphylium.[1]

The following is a brief description of the fungus:[2]

Stemphylium cucurbitacearum, n. sp.

The fungus produces spots ranging from 0.2 to 15 mm. in diameter upon leaves, rarely stems and petioles. The smaller spots vary from circular to angular in outline, with a light yellowish brown center and a darker reddish brown border, rarely white, on the upper surface; the spots are lighter on the lower surface. The larger spots vary from white to light brown, with reddish brown areas along the veins, giving a mottled appearance.

The mycelium is hyalin to light brown, vacuolate, septate, branched, growing readily on various culture media. Diameter, 2.5 to 10 μ. Fusion of the hyphæ is quite common.

[1] A specimen of this fungus was submitted to Dr. W. G. Farlow who verified the writer's conclusions in regard to its systematic position.

[2] **Stemphylium cucurbitacearum,** sp. nov.—Maculis in foliis, raro caulibus, 0.2 to 15 mm. in diameter; maculis minoribus orbicularibus vel angularibus, ad superficiem centro pallido-luteo-brunneis, rufo-brunneo-marginatis raro albidis, infra albidis; maculis largioribus albidis vel lacte-brunneis per venas rufis-fuscis; mycelio hyalino vel lacte-brunneis; septato, ramoso; sporophoris hyalinis vel lacte-brunneis 1–5 septatis, 10–30 × 7–12 μ, cellulis singulis deinde globosis; sporidiis muriformibus, subglobosis, atro-brunneis 25–50 μ diameter, e cellulis 5–20, 10–18 μ diameter, hypophyllis, in apice sporophororum singulis.

Habitat in foliis vivis, raro caulis, *Cucumeris sativi* et *Cucurbitae peponis*, Plymouth, Lapaz, Lakeville, Hamlet, et North Liberty, Indiana; et Bowling Green, Ohio, America Borealis.

Sporophores arise singly on the lower surface of the leaf as branches of the mycelium, hyalin to light brown, 1- to 5-septate, 10 to 30 by 7 to 12 μ, the individual cells eventually becoming globose and easily breaking apart, bearing a single spore at the apex.

The spores are nearly globose, dark brown, muriform, 25 to 50 μ in diameter, composed of 5 to 20 cells each of which are 10 to 18 μ in diameter, easily breaking away from the sporophores. They develop abundantly on the lower surface of leaves and on several of the ordinary culture media.

Habitat: Leaves, rarely stems and petioles, of *Cucumis sativus* and *Cucurbita pepo* (gourds and summer squash). The type specimen was collected from a cucumber field, near Plymouth, Ind., on September 27, 1915. Specimens were also collected from Lapaz and Lakeville, Ind., in September, 1915, and Bowling Green, Ohio, in October, 1915 (W. W. Gilbert); Hamlet and North Liberty, Ind., in September, 1916.

The type specimen has been deposited in the herbarium of the United States Department of Agriculture, Washington, D. C. Duplicates of this type material have been deposited in the herbaria of the New York Botanical Garden, New York, the Harvard Cryptogamic Laboratory, Cambridge, Mass., and the Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Ind.

CULTURAL CHARACTERS OF THE FUNGUS

The fungus has been grown on the following media: Corn-meal agar, beef agar, Lima bean agar, oat agar, potato-glucose agar, potato plugs, cucumber-stem agar, string-bean agar, and beef bouillon. On standard beef agar and in bouillon a limited growth with slight spore production occurs. On potato-glucose agar and string-bean agar abundant growth with abundant spore production takes place. The growth on the other media tried is intermediate between these two.

The growth on one series of string-bean-agar cultures will be given in detail as a typical example. These transfers were made on November 9, 1916, with spores from a 15-day-old culture.

November 11, 1916.—Growth was visible around point of inoculation as light radiating strands of mycelium.

November 14, 1916.—Growth around point of inoculation was rather granular for 2½ to 3 mm. in diameter. Light radiating growth around this was 6 to 8 mm. in diameter. Examination with a binocular microscope showed that the granular appearance was due to the beginning of spore formation. A few drops of liquid were present on mycelium in some tubes, owing to exudation.

November 17, 1916.—Growth was 12 to 14 mm. in diameter. A thin layer of brown spores 8 to 10 mm. in diameter, with granular-appearing mycelium, surrounded this. Around portions of the margin there was a narrow light flocculent growth where spore formation had not yet begun.

November 22, 1916.—Growth was 16 to 20 mm. in diameter. The surface was almost entirely covered with a heavy dark-brown or nearly black mass of spores. The spore formation was less abundant at the edge, or in places was absent. A few white tufts of mycelium 1 to 2 mm. in diameter had appeared over the spores.

December 1, 1916.—Heavy dark spore masses appeared in all the tubes, partly covered by a white fluffy growth of mycelium.

At first this fluffy growth of mycelium following heavy spore production was rather puzzling. Careful reisolation of all strains showed that it was not due to contamination. Further observations have shown that it arises from germination of the spores in the cultures.

RELATION TO LIGHT.—Sixteen tubes each of string-bean and potato-glucose agar were inoculated with two strains of the fungus, and one-half were kept in the dark, while the others were exposed to the ordinary light of the laboratory. No difference could be observed in their growth.

RELATION TO MOISTURE.—Growth was usually slower on media that had dried somewhat on the surface. It was also slower in a dry atmosphere than when the tubes were placed in a moist atmosphere under a bell jar or in the culture chamber. In the latter cases the mycelium exuded copious drops of liquid. Spore formation was usually retarded in a moist atmosphere.

RELATION TO TEMPERATURE.—Cultures on string-bean and potato-glucose agar were exposed to various temperatures. For room temperature, 18° to 23° C., the regular laboratory was used. For temperatures above this the cultures were kept in the incubator. For temperatures below room temperature two different cold rooms were employed. The temperature in these cold rooms was not entirely uniform, but did not vary over 3° or 4°. The optimum temperature for development and spore production was found to be from 18° to 25°. Above 30° and below 15° growth was very scant, and only a few spores were produced. This low optimum may explain the writer's failure to find infection in the cucumber fields around Plymouth during the summer of 1916 before the cooler weather of September had arrived. During midsummer the average temperature was much above the maximum for the growth of this fungus. The writer has had no success in securing infections where the temperature has been high continuously at the time of inoculation.

SPORE FORMATION

In artificial cultures spore formation may be so abundant that the contents of the mycelium are almost entirely used up. The conidiophore begins as an outgrowth of a mycelial cell (fig. 1, A, B, C). It is at first nonseptate, but a septum is soon laid down near the mycelium, and as growth proceeds, additional septa are formed, in extreme cases as many as eight being produced (fig. 1, C–F). The end cell, or in some cases the cell next to the end, now divides by a longitudinal septum (fig. 1, G–I). Other longitudinal divisions follow rapidly, and in a short time a globular head consisting of from 5 to 20 cells or in extreme cases even more is produced (Pl. 24, B, C; and fig. 1, M, N). Meanwhile, as the longitudinal divisions progress, the cells gradually assume a dark-brown color, caused by a pigment in the cell wall.

The cells of the conidiophore, which at first are compressed tightly together, gradually become globose and loosely attached to each other and to the spore (fig. 1, M, N).

SPORE GERMINATION

To observe spore germination, Van Tieghem cells and slides supported in petri dishes were used. In the latter case drops of liquid containing the spores were placed directly on the slide. The spores were scraped from the surface of the culture or leaf and filtered through fine silk bolting cloth to separate them from each other, and they were then dropped on the slides. With the Van Tieghem cells the spores were transferred directly to the covers from cultures.

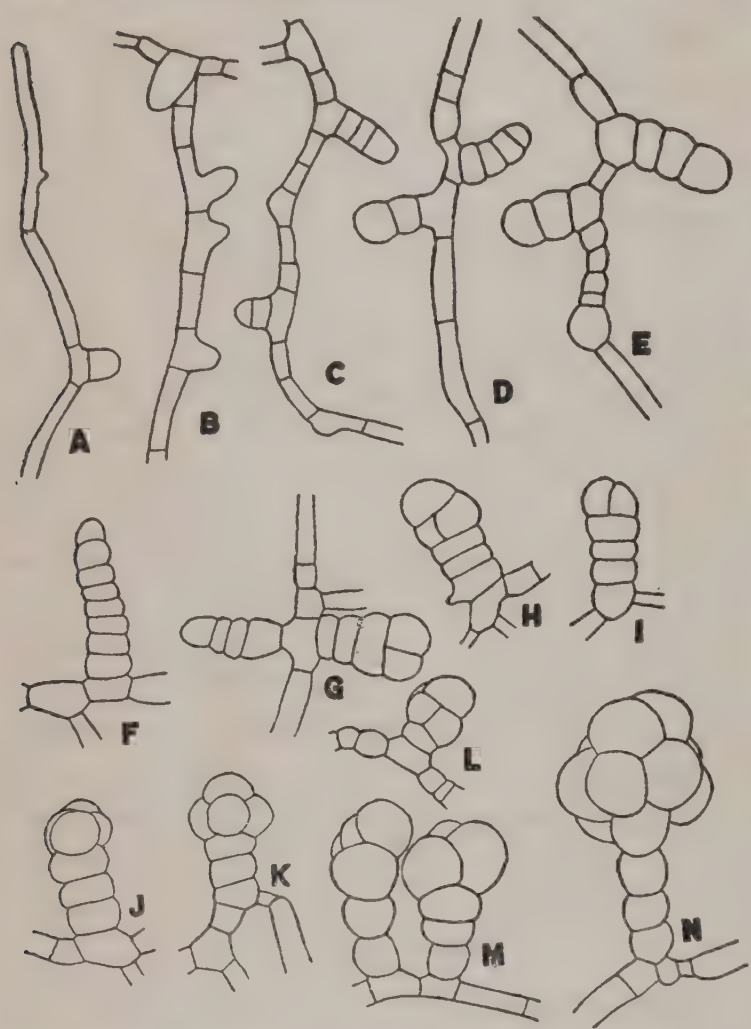


FIG. 1.—Spore formation of *Stemphylium cucurbitacearum* (Material taken from string-bean-agar cultures 12 days old. ×1,050): *A*, *B*, earliest stages of spore formation, showing origin of conidiophores on mycelium; *C–F*, early stages in spore formation, showing the transverse divisions of the conidiophores; *G–N*, various stages in the maturation of the spores.

The first signs of germination consist in a slight swelling of the spore owing to increased turgidity. In from three to six hours some of the cells were observed to have a slight protuberance, the beginning of the germ tube (fig. 2, A, F). Two hours later the germ tube was found to have pushed out 5 to 10 mm. (fig. 2, B, G). In beef bouillon or other nutrient bouillon the growth is fairly rapid from now on. In fig. 2, A–E, is shown a spore in various stages of germination. Not all of the cells germinate, but in the case of large spores the writer has observed as many as a dozen germ tubes. Only one germ tube is produced from a single cell. The germ tubes branch profusely and soon become septate (Pl. 24, A; fig. 2, E, H). Within 72 hours they have frequently become a tangled mass of threads. Fusions of the hyphæ are quite common (fig. 3).

In water, germination is slower, and growth soon ceases. Frequently vesicular, highly vacuolate, thin-walled bodies are produced (fig. 2, I, J). These may break away, or a germ tube may grow from them while still attached to the original germ tube. They probably have no significance in the life history of the organism.

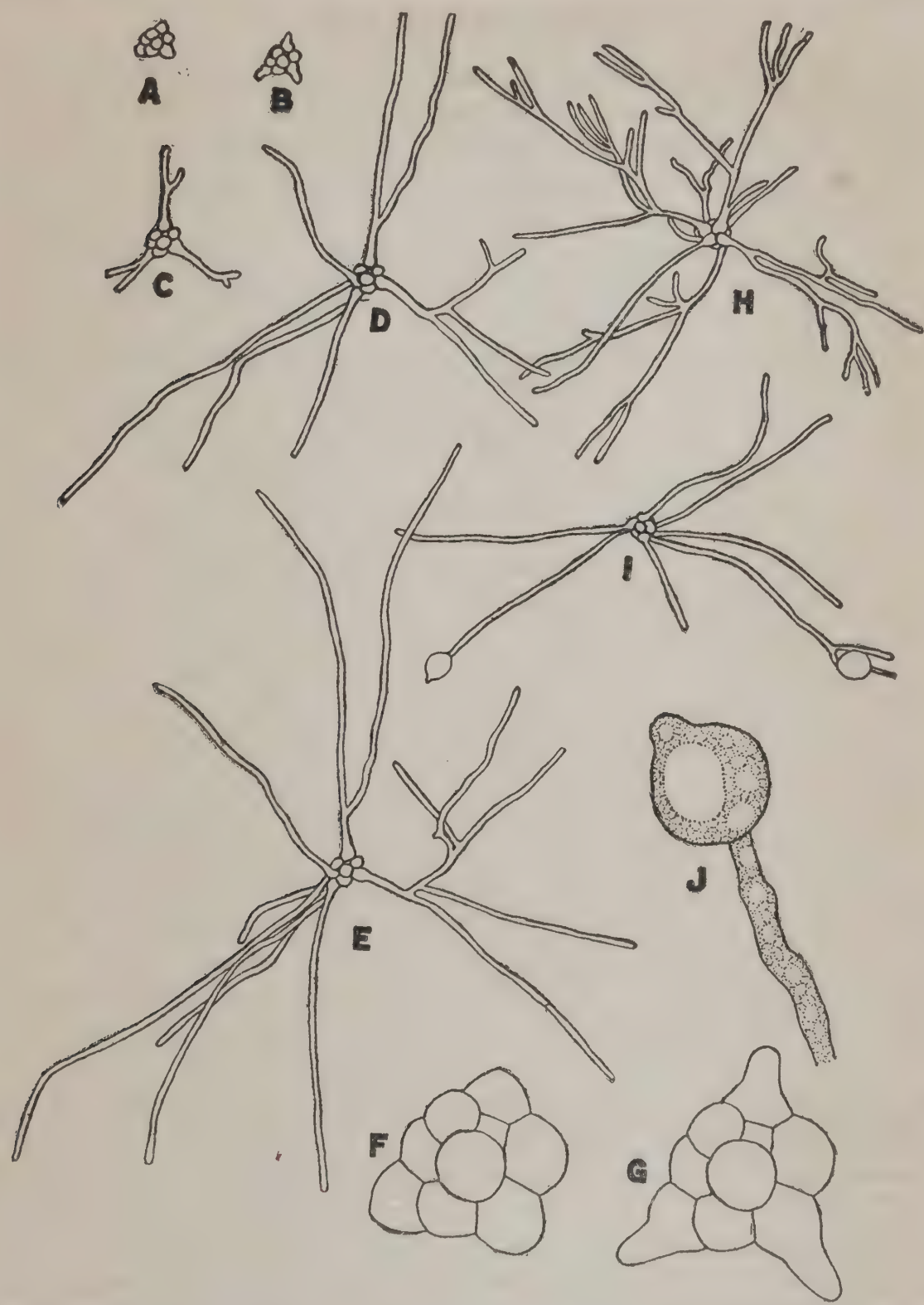


FIG. 2.—Spore germination of *Stemphylium cucurbitacearum: A–E*, various stages in the germination of a single spore in nutrient bouillon. *A*, 6 hours; *B*, 8 hours; *C*, 12 hours; *D*, 24 hours; *E*, 36 hours. ×105. *F–G*, same as *A–B*, but more highly magnified, showing origin of germ tubes. ×1,190. *H*, spore germinated in nutrient bouillon after 48 hours. ×105. *I*, spore germinated in distilled water after 48 hours, showing thin-walled vesicular bodies. ×105. *J*, portion of the germ tube shown in *I* more highly magnified. ×1,190.

The maturity of the spore has nothing to do with its capacity for germination. In Plate 24, A, is shown a germinating spore which has not yet begun to turn brown. The writer has secured germination from the earliest stages of spore formation.

LIFE-HISTORY STUDIES

The inoculation period for this fungus is usually from three to five days. Infection may occur either on the upper or lower surface of the leaves. In a few cases the writer has observed germ tubes entering through the opening of the stomata. Whether this is the only method of infection was not determined. For observing this phenomenon pieces of inoculated tissue were fixed and stained with eosin or sectioned and stained with Fleming's triple stain. The mycelium penetrates the tissues of the leaf, causing them to collapse somewhat. In from 8 to 12 days spore formation is found to be taking place on the lower surface of the leaf. The conidiophores arise from mycelium growing on the surface.

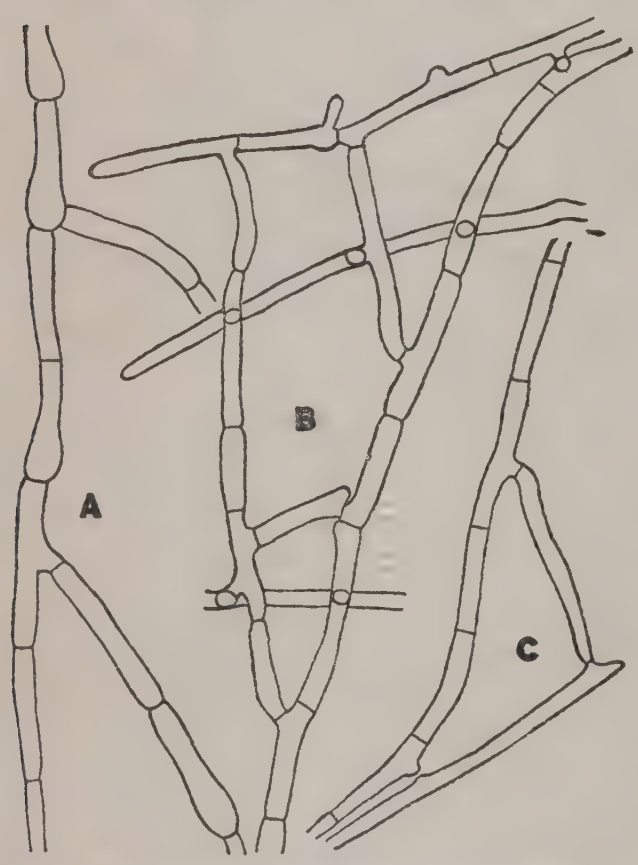


FIG. 3.—Mycelium of *Stemphylium cucurbitacearum: A*, Mycelium from a string-bean-agar culture 12 days old. ×1,050. *B–C*, Mycelium from spores germinated in nutrient bouillon, 72 hours old, showing fusions of the hyphæ. ×1,190.

The fungus appears to overwinter as mycelium in the tissues of the host. Whether the spores may also live over a winter has not been definitely determined. Viable spores were obtained from both cultures and leaves that were exposed outside over a winter, but it was impossible to determine whether these spores had overwintered or had been produced in the spring. Spores taken from culture media and placed outside in paper packets failed to germinate in the spring.

The spores may be scattered by wind, rain, insects, animals, or garden tools. When slightly wetted, the spores have a tendency to stick together, owing apparently to surface tension phenomena. This would allow them to be easily carried by insects or animals. The spores may be easily spattered about by raindrops. To test this point, the writer inoculated the upper leaves of three plants; and as soon as spore formation occurred, the plants were sprayed with water. In a few days infection was general on the lower leaves, while healthy plants sprayed as controls remained free from disease. The spores may also be carried short distances at least by strong winds. The writer has caught spores on the moistened slides several inches from leaves held in the current of an electric fan.

CONTROL OF THE FUNGUS

Certain preliminary experiments give promise that this disease may be easily controlled by spraying the leaves with Bordeaux mixture.

In the laboratory the slides were sprayed in the manner outlined by Wallace, Blodgett, and Hesler.[1] The method was as follows: One end of a glass slide was sprayed with the spray mixture, and after drying, the slide was supported on glass slips in a petri dish. A suspension of spores in water evenly distributed by filtering through silk bolting cloth was placed on both the sprayed and unsprayed ends of the slide. In Table II is given the percentage of germination from the various treatments.

TABLE II.—*Percentage of the spore germination of Stemphylium cucurbitacearum on slides after spraying with Bordeaux mixture*

Strength of Bordeaux mixture.	April 30.		May 10.		May 12.		May 15.		Average.	
	Sprayed.	Control.	Sprayed.	Control.	Sprayed.	Control.	Sprayed.	Control.	Sprayed.	Control.
1–2–50....	25	63	3	77	14	70	19	85	15	74
2–4–50....	12	63	3	75	4	62	10	90	7	72
3–6–50....	3	66	Tr.	79	1	66	4	80	2	73
4–6–50....	Tr.	63	Tr.	76	Tr.	63	Tr.	82	Tr.	71
5–5–50....	1	61	0	70	Tr.	70	Tr.	82	Tr.	71

In each case the figures given represent the average of three slides. Germination is recorded regardless of whether the germ tube was long or short. In many cases, especially where the higher strengths of Bordeaux mixture were used, the germ tubes were so short that infection could hardly have taken place. For example, on the slides sprayed with Bordeaux mixture 1–2–50 the germ tube averaged about 40 μ, while on the controls they were several hundred microns in length. Consequently the figures given in Table II may underestimate rather than overestimate the value of the spray mixture, provided the leaves are thoroughly and evenly coated. To judge from the results given in Table II, Bordeaux mixture 3–6–50 would appear to be entirely efficient against this disease.

To test this point further, four plants were sprayed with Bordeaux mixture 2–4–50 and 3–6–50, respectively. The next day these plants were inoculated with a suspension of spores from a potato-glucose-agar culture. Four plants sprayed with water were inoculated as controls. Two small spots developed on the plants sprayed with Bordeaux mixture 2–4–50, the other plants sprayed with the mixture remaining healthy. The control plants developed numerous infections.

[1] WALLACE, Errett, BLODGETT, F. M., and HESLER, L. R. STUDIES OF THE FUNGICIDAL VALUE OF LIME-SULPHUR PREPARATIONS. N. Y. Cornell Agr. Exp. Sta. Bul. 290, p. 167–174. 1911.

In addition to spraying, certain sanitary measures should be adopted. Diseased vines should be destroyed, if possible, in the fall. Crop rotation should be practiced. Vines should not be disturbed when wet with dew or rain.

SUMMARY

During the summers of 1915 and 1916 the attention of the writer was called to a peculiar leafspot on cucumber that was doing more or less damage to cucumber fields in the vicinity of Plymouth, Ind., and Bowling Green, Ohio. The spots vary in diameter from 0.2 to 15 mm. The small spots, ranging from 0.2 to 3 or 4 mm. in diameter, may be circular or angular in outline. The center is light yellowish brown, surrounded by a reddish-brown border; rarely, the spot is nearly white. The larger spots are usually nearly white or tinged with brown, with reddish-brown areas along the veins and frequently with brownish centers, giving the spots a mottled appearance. From these spots a fungus belonging to the genus Stemphylium has been isolated. The parasitism of this fungus to the leaves of cucumbers, gourds, and squashes has been repeatedly demonstrated by successful inoculations. Four varieties of cucumber, two of gourd, and two of squash have been successfully inoculated. So far as ascertained, no data regarding this disease has been published hitherto, and the causal organism is described as a new species under the name "*Stemphylium cucurbitacearum.*"

Cultural work with this fungus has shown that high temperatures and a dry atmosphere are unfavorable to its development. Consequently serious trouble may be expected only in cool weather, especially when combined with abundant moisture.

The fungus lives over the winter in or on the diseased vines. The spores are disseminated by wind, rain, insects, etc.

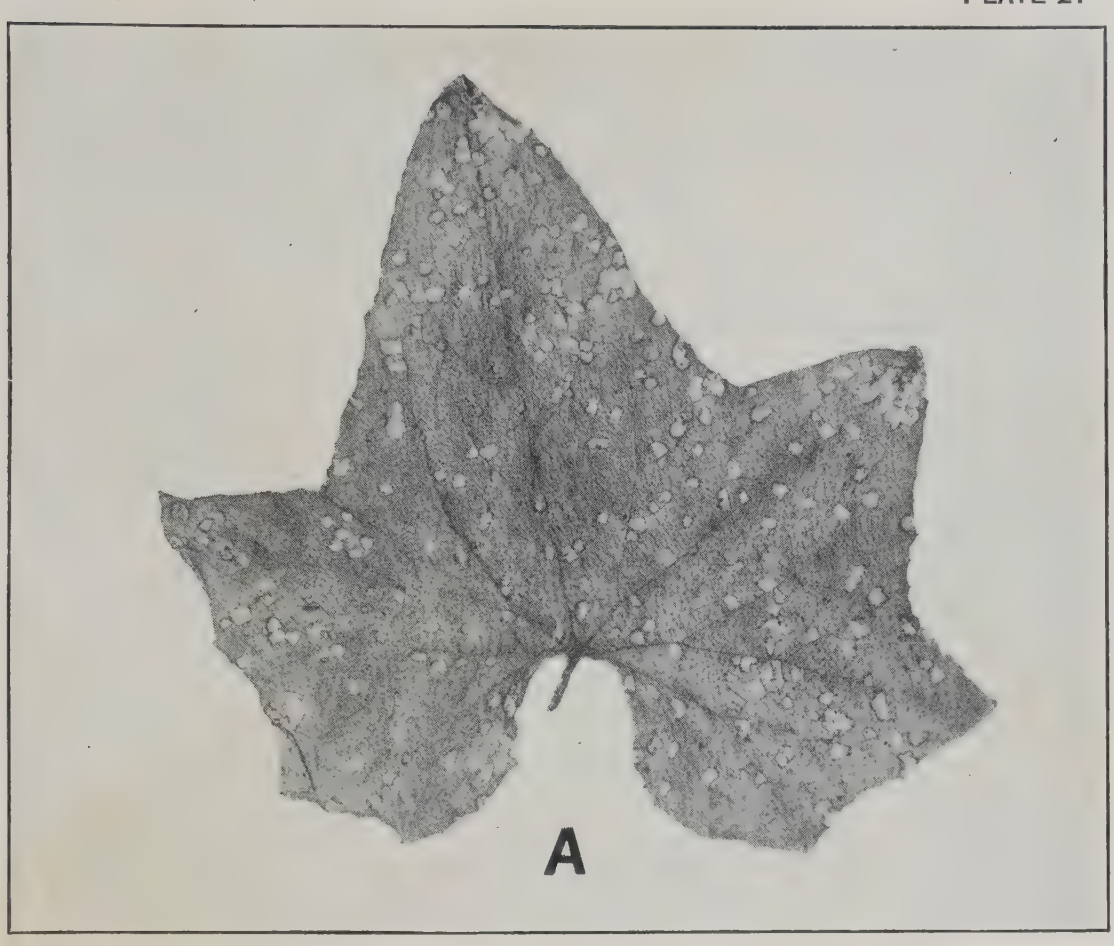
Preliminary experiments have given promise that this disease may be controlled by Bordeaux mixture. In addition to this, sanitary measures such as the destruction of diseased vines and crop rotation, as well as the avoidance of work among plants when wet, should be practiced.

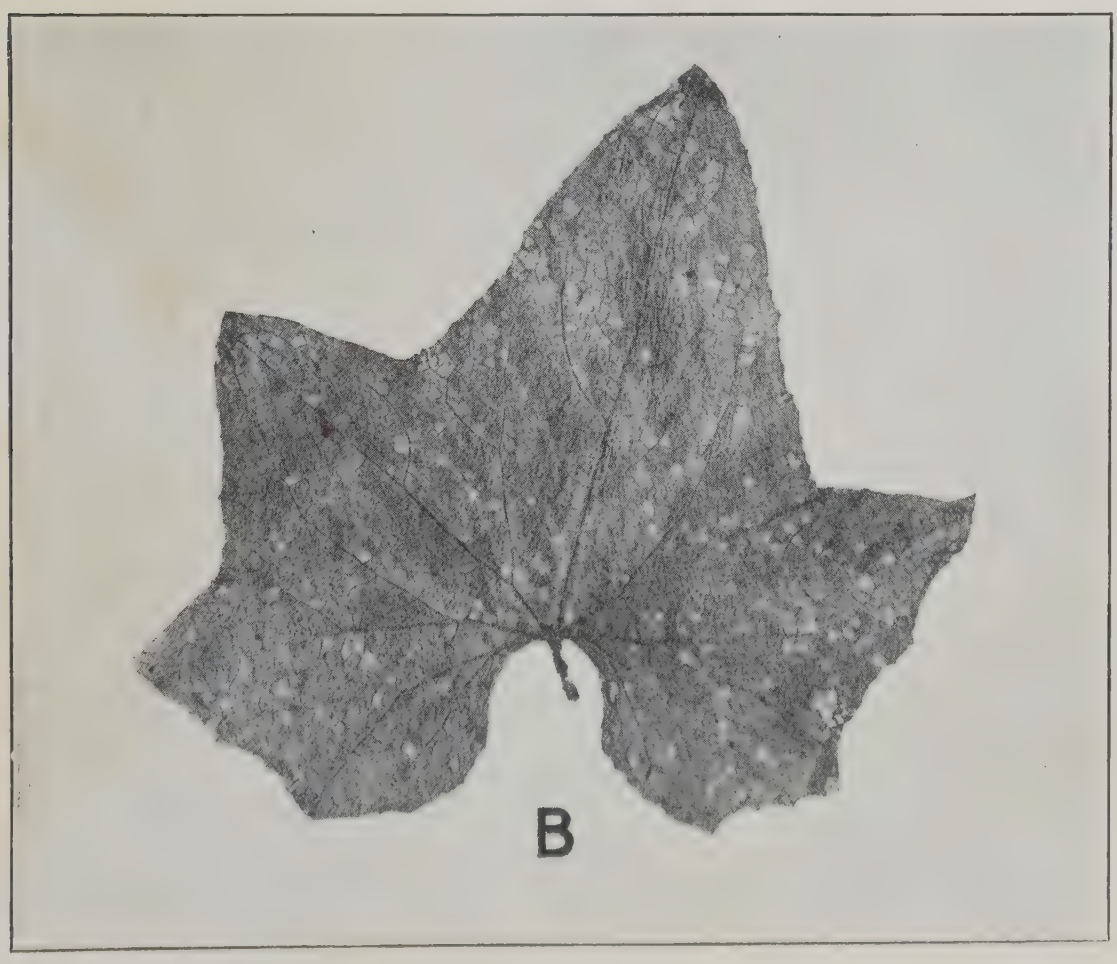
PLATE 21

Stemphylium cucurbitacearum

A.—Small spots on upper surface of leaf with a few larger spots formed by coalescence of small spots.

B.—Same leaf as shown in A, but lower surface view.


A


B

A

B

PLATE 22

Stemphylium cucurbitacearum

A.—Large and small spots on upper surface of cucumber leaf.

B.—Large spots on cucumber leaf showing brown centers surrounded by lighter area.

PLATE 23

A.—Early stage of the Stemphylium leafspot on cucumber from artificial inoculation, showing the formation of the large mottled spots.

B.—Early stage of the Stemphylium leafspot on gourd from artificial inoculation, showing very fine spots in groups.

A

B

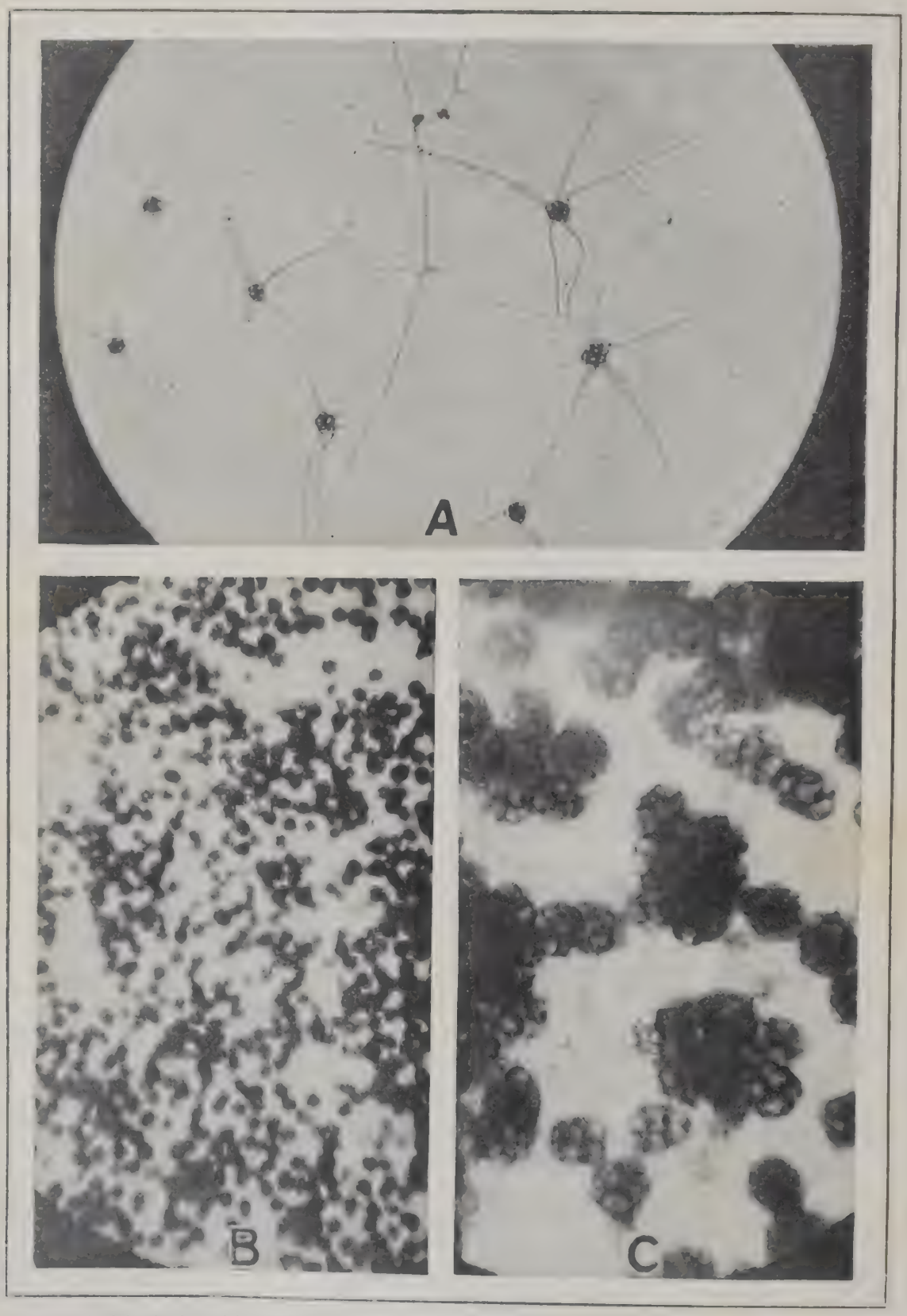

A
B
C

PLATE 24

Spore formation and germination of *Stemphylium cucurbitacearum*

A.—Spores germinating in tap water. ×95.
B.—Spore formation in string-bean-agar culture 15 days old. ×60.
C.—Portion of B more enlarged. ×225.

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YELLOW-LEAFBLOTCH OF ALFALFA CAUSED BY THE FUNGUS PYRENOPEZIZA MEDICAGINIS[1]

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Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In the spring of 1914, when the writer began a study of the foliage diseases of alfalfa (*Medicago sativa*) in the vicinity of Madison, Wisconsin, special attention was given to the leafspot caused by the fungus *Pseudopeziza medicaginis*, which is generally regarded as the most important disease of this plant. But it soon became apparent that another disease, which had not previously been mentioned as occurring in the United States, was responsible, under certain conditions at least, for even greater damage to the crop than the well-known leafspot. A study of this disease was undertaken in addition to the work on leafspot, and a brief report was made in 1916 (*10*).[2] Since that time the work on this disease has been continued, stimulated by an increasing recognition of its importance. The results are presented in the following pages.

THE DISEASE

DISTRIBUTION

During the two summers which have elapsed since the yellow-leafblotch was first noted in the United States, there has not been opportunity to make a complete survey of its distribution. It has been collected by the writer or correspondents in Vermont, New Jersey, Virginia,

[1] This paper presents one part of the results of a study of a group of alfalfa and clover diseases, which was begun in the Department of Plant Pathology at the University of Wisconsin in 1914. In 1916 the writer became a collaborator of the Office of Cotton, Truck, and Forage Crop Disease Investigations, of the Bureau of Plant Industry, and through cooperation between the Bureau and the Wisconsin Agricultural Experiment Station, the scope of the work was extended. The work has been done under the immediate direction of Dr. L. R. Jones, to whom grateful acknowledgments are expressed. Other members of the Department of Plant Pathology at the University of Wisconsin have contributed many valuable suggestions, and several correspondents have furnished specimens and information which have added greatly to the value of the work.

[2] Reference is made by number (italic) to "Literature cited," p. 329.

New York, Ohio, Kentucky, Tennessee, Wisconsin, Minnesota, Iowa, Kansas, South Dakota, California, Idaho, Oregon, and Washington. To judge from the wide range of conditions under which the disease has already been found, there appears to be no reason to doubt that it will be found in practically all of the important well-established alfalfa-growing regions in the country. It is possible that this leafblotch is not yet introduced into some of the newer and more isolated districts.

Outside the United States the only record of its occurrence in America is from Argentina (5). In Europe the disease has been found in Austria, Germany, France, and Italy, where it has long been known, though not regarded as important.

ECONOMIC IMPORTANCE

To what extent the yellow-leafblotch is a serious economic factor in the culture of alfalfa it will be impossible to state with confidence until a larger amount of data has been collected over a period of years; but that it does produce large loss of foliage under certain conditions is beyond doubt. From observations made largely at Madison, Wis., it appears to vary widely in the severity of its attack on different cuttings from the same field, depending largely on the abundance of infectious material and on weather conditions favoring infection. Under favorable conditions it has caused much larger loss of foliage than the leafspot caused by the fungus *Pseudopeziza medicaginis*. For instance, when the first crop was being cut on the experiment station farm at Madison, on June 19, 1916, it was estimated by observers that this disease had destroyed about 25 per cent of the foliage, and that at least 75 per cent of that remaining was more or less infected. In the summer of 1915 the disease was twice reported in private correspondence to the writer as of economic importance, by Dr. M. P. Henderson in the Rogue River Valley, Oreg., and by Mr. L. E. Melchers in Kansas. In a recent report (*11*) Melchers has stated that in many places in Kansas in 1916 the disease caused a loss of 40 per cent of the foliage of the first and second crops.

Frequently only a small part of the diseased foliage falls off and is lost. The fungus does not usually kill the invaded tissue until a large part of the leaf has been penetrated; but invaded tissue easily becomes water-soaked during rains, whereupon the leaf dies, and upon the return of dry weather it quickly shrivels and falls.

DESCRIPTION (PL. D)

Although the disease varies considerably in appearance, it has characteristics which make it easily distinguishable from other alfalfa diseases. The first visible evidence of its presence on the leaf is usually a blotch of characteristic yellow color, with its longer diameter parallel to the

direction of the veins. In a few days the blotch may extend from the midrib to the margin if the leaf is small. In shape it remains somewhat elongate, as though restricted by the veins. It does not appear that an entire leaflet is ever invaded by a single infection. In color it becomes a deeper yellow, often approaching a brilliant orange on the upper surface, and a little paler beneath.

Shortly after the appearance of the yellow blotch, sometimes at its first appearance, the central portion of the area shows on the upper surface of the leaf small orange-colored points which indicate the location of pycnidia. They may be very inconspicuous at first. They may be closely grouped along the center of the blotch if it is small, or may be more scattered if it is large. A smaller number of pycnidia usually emerge later on the lower side of the leaf. They soon become deep brown, or even almost black. On mature foliage which has lost its deep-green color, and especially upon plants with narrow leaflets, these elongate groups of dark-colored pycnidia may be the most easily recognized characteristic of the disease.

The diseased areas may not die and dry out for a long time, especially the foliage may take on a rusty-yellow color with no very distinct lesions, but with pycnidia scattered more or less uniformly over the leaf. Under very dry conditions such leaves may dry out quickly with little change in color. But during protracted rains they quickly become water-soaked and fall off.

Under very favorable conditions of protracted cool weather, especially in the autumn, these diseased areas may show on the lower surface of the leaf a number of small black bodies which may develop into apothecia before the death of the entire leaf. Ordinarily apothecia do not develop until after the death of the entire leaf (Pl. 26). At this time the diseased areas become very dark brown or black in color, in contrast to the light-brown color of that part of the leaf which was not diseased. If the dead leaves remain attached to the plant, they often show a tendency to curl spirally with the lower surface outward. The first apothecia appear as small black dots, rarely as much as a millimeter in diameter, scattered along the diseased areas on the lower surface of the leaf. Later, a few may appear on the upper surface. Occasionally newly infected leaves which have been killed prematurely by frost may develop scattered apothecia which are not located on blackened areas; but if the disease has been at all abundant and the plants have not been cut close too late in the fall, there are usually a sufficient number of dead leaves with characteristic blackened areas present to make possible the recognition of the presence of the disease even during the winter months.

The disease occurs on stems as well as leaves, but not as abundantly. From field observations it appears that the fungus usually requires a longer time to produce a conspicuous lesion on the stem than on the

leaf. Lesions appear as elongate yellow blotches, which soon turn a dark chocolate-brown.

The killed area rarely girdles the entire stem. Pycnidia are not as abundant as on the leaf. Apothecia have been found on stems but once, and then not well developed or abundant. No case has yet been observed where a plant has appeared to be seriously affected by the presence of this disease on the stem.

THE CAUSAL ORGANISM

TAXONOMY

In the previous report (*10*) the writer expressed the opinion that the fungus causing the yellow-leafblotch of alfalfa was *Phyllosticta medicaginis* (Fuckel) Sacc. (originally described as *Ascochyta medicaginis* Fuckel; *4*), and that experimental evidence indicated that this species of Phyllosticta is the conidial stage of *Pyrenopeziza medicaginis* Fuckel, which appears later on the dead leaves of this host. Additional experimental evidence has confirmed this opinion.

Shortly after this report had been made, an earlier name applied to the conidial stage of this fungus was found. A comparison of the descriptions indicated that *Phyllosticta medicaginis* was identical with a fungus which had previously been described by Desmazieres under the name "*Sporonema phacidioides*" (*3*). A comparison of the type specimen of *S. phacidioides* [1] with the type specimen of *Phyllosticta medicaginis* [2] was kindly made by Dr. W. G. Farlow. He decided that the fungi to which the two names were applied were identical, and that the names should therefore be regarded as synonyms.

In addition to the names already mentioned, "*Gloeosporium morianum* Sacc." has been suggested by Von Höhnel (*6*) as belonging to this synonymy. From an examination of the description of *G. morianum* it appeared to the writer that Von Höhnel was probably correct. Therefore specimens of *Sporonema phacidioides* were sent to Dr. P. A. Saccardo with the request that they be compared with the type specimen of *G. morianum*. Dr. Saccardo kindly made the comparison and assured the writer that they are identical. Thus, the synonymy of the conidial stage of the fungus is as follows:

Sporonema phacidioides Desm., 1847, Ann. Sci. Nat. Bot., s. 3, t. 8, p. 172–192.

Ascochyta medicaginis Fuckel, 1869/70, Jahrb. Nassau. Ver. Naturk., Jahrg. 23/24, p. 388.

Phyllosticta medicaginis (Fuckel) Sacc., 1884, Syll. Fung., v. 3, p. 42.

Gloeosporium morianum Sacc., 1892, Syll. Fung., v. 10, p. 458.

[1] Desmazieres, J. B. H. J. PLANTES CRYPTOGAMES DE FRANCE. fasc. 33, no. 1645. Lille, 1847.

[2] Fuckel, Leopold. FUNGI RHENANI EXSICCATI. no. 488. 1863.

The first description of the ascigerous stage of *Sporonema phacidioides* was given by Fuckel in 1870 (*4*) under the name "*Pyrenopeziza medicaginis.*" No record of any other collection of this fungus than Fuckel's type collection[1] has been found. Since *Pyrenopeziza medicaginis* Fuckel is the name of the ascigerous stage, it is the name which should be applied to the fungus as a whole, according to present rules of nomenclature.

The fact that *Sporonema phacidioides* is found to be the conidial stage of *Pyrenopeziza medicaginis* is a matter of considerable interest, because this fungus has long been regarded as a possible conidial stage of the leafspot fungus *Pseudopeziza medicaginis*. This misconception appears to have developed in the following manner. When, in 1847, Desmazieres described *S. phacidioides*, making it the type species of the new genus (*3*), he noted that the pycnidia opened by irregular valves in much the same manner as the apothecia of *Pseudopeziza medicaginis* (then known as *Phacidium medicaginis*), and that it occurred in association with this species of Phacidium. This common morphological feature and the association of the fungi suggested the species name "*phacidioides.*" Next, in 1865, during the course of a discussion of *Pseudopeziza medicaginis*, Tulasne (*14*) made a note of the fact that the two fungi occur together. Although from a careful study of Tulasne's statement it does not appear that he intended to indicate more than an incidental association of the two fungi, yet subsequent writers generally have interpreted it as pointing out a probable relationship. For instance, Brefeld (*1*) says:

> Von ihr [*Pseudopeziza medicaginis*] giebt Tulasne Pycnidien mit kleinem länglich eiformigen Sporen an, die in ahnlicher Weise wie die Ascusfruchte ihre Hymenium basslegen sollen.

Thus, scattered through the literature of *Pseudopeziza medicaginis* from the time of Tulasne's statement, are found, chiefly in European literature, a large number of references to the conidial stage of this fungus which clearly indicate that *Sporonema phacidioides* was referred to, although it was not always specified by name. The persistence of the idea that *S. phacidioides* is the conidial stage of *Pseudopeziza medicaginis* is all the more surprising, in view of the fact that there appear to have been no recent collections of the former on alfalfa, and all the older collections were from France and Belgium only.

The reason for the scarcity of collections of *Sporonema phacidioides* is obviously as follows: In 1870 Fuckel (*4*) had described what now appears to be the same fungus under the name "*Ascochyta medicaginis.*" In 1884 Saccardo (*13*) transferred the species to the genus Phyllosticta. Thus, almost all the collections of this fungus outside of France and Belgium were placed under one or the other of these two names. And

[1] Fuckel, Leopold. FUNGI RHENANI EXSICCATI. no. 1594. 1865.

curiously enough, although the fungus was widely known under these names, no one appears to have suggested a possible relationship of the fungus under this name with *Pseudopeziza medicaginis* as had been suggested for the same fungus under the name "*Sporonema phacidioides*."

Under the name "*Gloeosporium morianum*," by which it was described by Saccardo in 1886 (*12*), the fungus does not appear to have become widely known. Only a single subsequent reference to an identification of the fungus by that name (*2*) has been found.

Thus far the writer has traced the development of the misconception of *Sporonema phacidioides* as the conidial stage of *Pseudopeziza medicaginis* on alfalfa. This misconception appears to have gone even farther, and to have been responsible for three references to Sporonema on clover (*Trifolium pratense*) as the conidial stage of *Pseudopeziza trifolii*. The first of these is in a note over the signature of G. Von Niessl appended to No. 2057 of Rabenhorst's Fungi Europaei.[1] This collection is designated "*Pseudopeziza trifolii* (Bernh.) Fckl. St. *conidiophorus*." At the end of the note describing this fungus occurs this sentence:

> Das ganze Gebilde entspricht der alten Gattung Sporonema.

The other references to species of Sporonema on clover are by Jaczewski (*7*, *8*, *9*). In the second reference *Sporonema phacidioides* is specifically stated as occurring in Russia as the conidial stage of *Pseudopeziza trifolii*.

Since field observation and inoculation experiments both indicate that Sporonema does not occur on clover, it is a matter of interest to know what fungus on this host has been called by that name. Through the kindness of Prof. Thaxter, the writer has been permitted to study a portion of Von Niessl's collection in the Harvard herbarium. The fungus found here appears to be identical with *Gloeosporium trifolii* Pk. Owing to war conditions, it has not been possible to obtain specimens of the fungus which Jaczewski regards as the conidial stage of *Pseudopeziza trifolii*. For the present the writer can only assume tentatively that it is the same fungus that is given under that designation by Von Niessl. Since the general character of *Gloeosporium trifolii* is not greatly different from that of *Sporonema phacidioides*, it is not difficult to see why Von Niessl and Jaczewski, holding *Sporonema phacidioides* as the conidial stage of *Pseudopeziza medicaginis* on alfalfa, should have come to regard this species of Gloeosporium as the corresponding conidial stage of *Pseudopeziza trifolii* on clover.

MORPHOLOGY

MYCELIUM.—The mycelium within the leaf tissue varies greatly in diameter. Many of the lateral branches are very small, while older hyphæ may become greatly swollen. Hyphæ are found largely within, but

[1] Rabenhorst, G. L. FUNGI EUROPAEI EXSICCATI. new ed., cent. 21, no. 2057. Dresdae, 1876.

sometimes between the cells. When the tissue is thoroughly invaded, the interior of the larger palisade cells and many other large parenchyma cells become lined with a thick-walled cellular fungus layer. Finally the leaf tissue almost disappears and becomes replaced with a fungus stroma.

In culture the mycelium develops largely in the substratum, where it forms a dense mat that does not appear to amalgamate into a stroma, though in old cultures it may produce a stroma at the surface of the culture medium. The aerial mycelium usually appears as a close felt of hyphæ arising from the submerged mycelium. In case the mycelium reaches a height of about 2 mm., it tends to interweave into numerous little flexuous spires.

CONIDIAL STAGE.—As soon as the mycelium has thoroughly invaded the leaf tissue, conidia begin to appear in cavities among the palisade cells

FIG. 1.—*Pyrenopeziza medicaginis:* Advanced stage of development of the conidial stage.

just below the epidermal layer of the upper surface of the leaf. At first these cavities are nearly spherical in form, but they rapidly increase in size, extending for the most part in the direction parallel to the surface of the leaf (fig. 1). Thus they become greatly broadened, and often much lobed and convoluted in cross section. The lining of the cavity consists of interwoven hyphæ or of a very thin layer of fungus tissue from which the conidiophores arise in a closely packed layer. Next to the epidermis there may be a few large, somewhat circular, dark-colored cells which do not form a complete protective layer. There is no regularly developed ostiolum, the opening being merely irregularly torn through the epidermis. Occasionally when the cavity is large and the weather is moist, the torn ends of the ruptured epidermis become recurved, which exposes the layer of conidiophores (Pl. 25). When the leaf tissue

has become largely replaced with the fungus stroma, it shrinks somewhat in thickness, and the large groups of conidiophores sometimes appear to be at the surrounding leaf surface or even raised above it.

The conidiophores have a characteristic bottle shape, measuring about 12 to 14 μ long and about 3 μ in diameter at the base, but much smaller in the upper third of the length (fig. 2).

The conidia vary in shape and size. The longer conidia are nearly cylindrical, very slightly bent, and with rounded ends. The shorter conidia often appear slightly shrunken at one end. They measure 5 to 9 by 2 to 3 μ, the larger part being 6 to 7 by 2.5 μ.

The larger part of the conidia found in cultures are borne in acervulus-like structures at the surface of the substratum or in spherical cavities within the substratum. The base of the acervulus, or the wall of the cavity, consists of closely woven hyphæ usually made up of short, rounded cells from which the conidiophores arise. But in addition conidia may be abstricted terminally, or even laterally, from hyphæ at any point on the surface of a culture; or hyphæ may give rise to either isolated or small groups of conidiophores which produce the spores (fig. 3). Sometimes it appears that an acervulus develops from a nucleus of a few conidiophores arising in a group. For instance, cultures from ascospores discharged on alfalfa stems sometimes show all gradations between a few scattered conidiophores, a larger, more matted group, and a somewhat raised acervulus. Spores may be produced from acervuli in such numbers that they exude in milky drops. The spores are indistinguishable from those produced on the leaf, except that occasionally somewhat larger individuals can be found, measuring up to 10 μ in length.

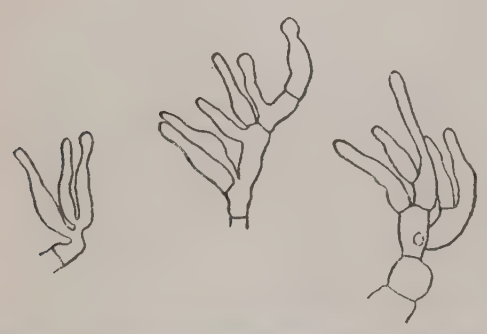

FIG. 2.—*Pyrenopeziza medicaginis:* Conidiophores from an alfalfa leaf.

In addition to what may be regarded as the normal conidia in culture, conidia-like structures are often formed on mycelium from ascospores discharged on agar to which little or no nutrient material has been added. The conidia-like structures are found submerged in the substratum, and are distinguished from normal conidia by the ovoid shape, slightly larger size, and by the fact that they are borne in groups (fig. 4).

ASCIGEROUS STAGE.—After the leaf tissue has been killed, when favorable conditions for further development of the fungus occur, small black stromatic masses emerge from the lower surface of diseased areas opposite the pycnidia. Later, they may appear in smaller number on the upper surface of the leaf, scattered somewhat beyond the black area on both surfaces. These black stromatic masses develop into apothecia. Before opening, the apothecium contains only a mass of vertical hyphæ with their upper ends free, and from these ends a few conidia may be

abstricted. When the apothecium is ready to expand, asci appear among the paraphyses, and the ascospores are soon mature and ready to be discharged (fig. 5).

The apothecia are sessile, 0.25 to 1 mm. in diameter, rarely larger. The outer wall appears black and is made up of thick-walled cells. The disk of the apothecium appears pale gray when expanded. The stromatic layer from which the asci arise is continuous with the stroma in the leaf, but it is composed of smaller cells rich in protoplasmic contents.

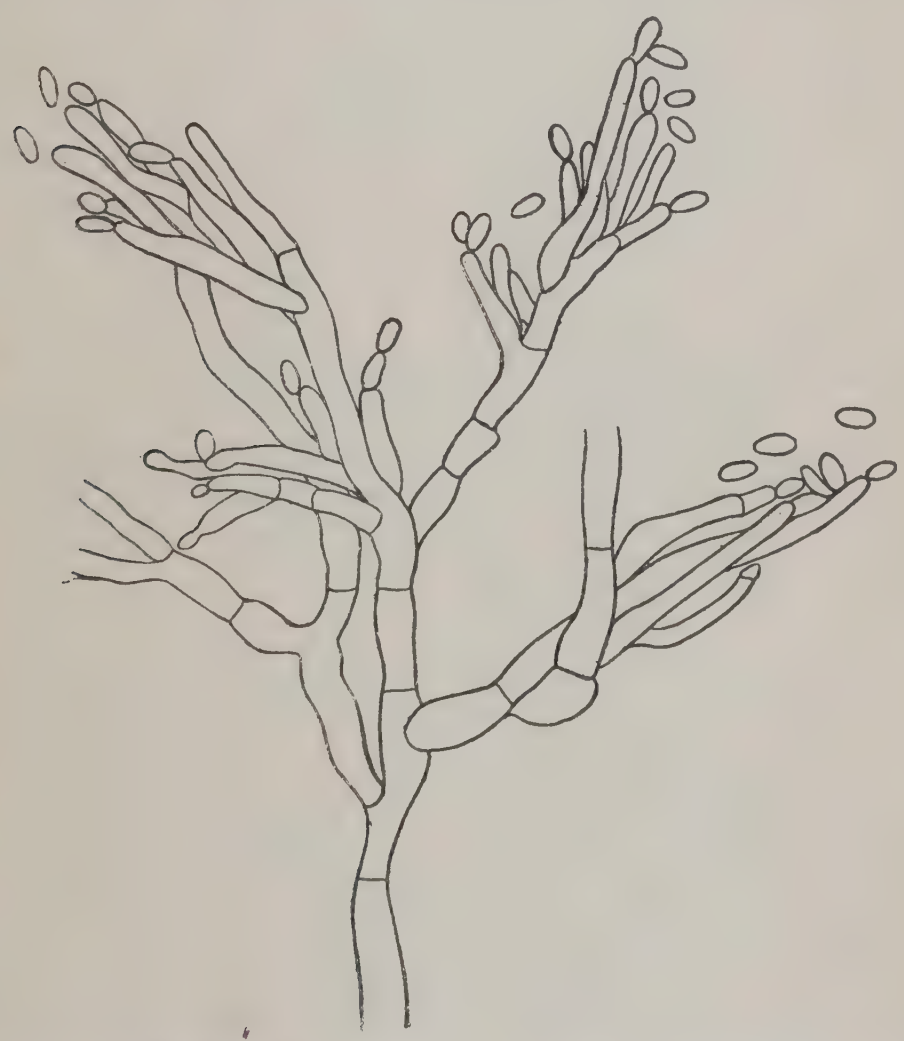

FIG. 3.—*Pyrenopeziza medicaginis:* Conidiophores from a culture at an early stage in the formation of an acervulus.

The paraphyses measure 50 to 80 by 2.5 to 3 μ. Occasionally they branch, in which case a septum may occur. The asci measure 60 to 75 by about 10 μ. The ascospores are slightly ovoid in shape and measure 8 to 11 by 5 to 6 μ. By far the largest number are 9 to 10 μ long.

PHYSIOLOGY

Isolation of the Fungus

For over a year after the yellow-leafblotch had first been observed, all efforts to isolate the fungus which appeared to be the cause were unsuccessful. All attempts to germinate conidia failed. Attempts to plate out

diseased fragments of the leaf after surface sterilization were unsuccessful at that time because bacteria or saprophytic fungi, which had entered the diseased tissue at a very early stage, quickly overran the slow-growing pathogen in culture. However, in October, 1915, the disease appeared on clean, vigorous plants in the greenhouse. Platings from these leaves gave the first success in isolation that was achieved. The fungus thus obtained grew very slowly, and produced conidia indistinguishable from those found on the diseased leaves. Like the conidia found on diseased leaves, these failed to germinate, and did not produce the disease when sprayed on alfalfa plants. From this time on nearly all the successful isolations have been made from plants in the greenhouse. However, late in the autumn of 1916, successful isolations were made from vigorous plants in a field near the laboratory where the progress of infection

FIG. 4.—*Pyrenopeziza medicaginis:* Conidia-like structures which occasionally develop on mycelium from germinating ascospores.

could be watched and isolations made at the time of the first visible stages in the development of the disease.

The method followed in making these isolations was as follows. The diseased area at an early stage of development was cut from the leaf. The fragments were small—not more than 5 mm. long—and they were cut in such a way that the diseased area was not entirely surrounded by healthy tissue. Much time is required by the fungus in culture to cross even narrow bands of healthy tissue which may separate it from the surface of the substratum. The leaf fragment was then dipped into 50 per cent alcohol, and sterilized on the surface in a mercuric chlorid solution for from 1 to 1½ minutes. After washing, the fragments were placed singly on slopes in test tubes. Petri dishes are unsatisfactory, since they usually dry out before the end of the three weeks that are required for the fungus to grow sufficiently to furnish transfers. The culture medium most favorable for the development of mycelium appears to be potato agar.

After the ascogenous stage of the fungus had been found, efforts were made to make isolations from ascospores. But no practicable method

of obtaining cultures free from bacteria from ascospores from apothecia on dead leaves was devised. When plated out in agar, the ascospores refuse to germinate. When discharged on an agar surface, germination was not vigorous and bacteria were almost always present. A few germinating single spores were obtained free from bacteria, but they did not develop into cultures. In fact, no cultures from single spores of this fungus have yet been obtained.

Later, however, a method of obtaining apothecia under conditions of pure culture made it possible to secure cultures from ascospores. While isolations from the diseased plants in the greenhouse were being made repeatedly to determine if the fungus which had been obtained was constantly associated with the disease, various culture media were tried out. In the course of these experiments it was found that when agar with no

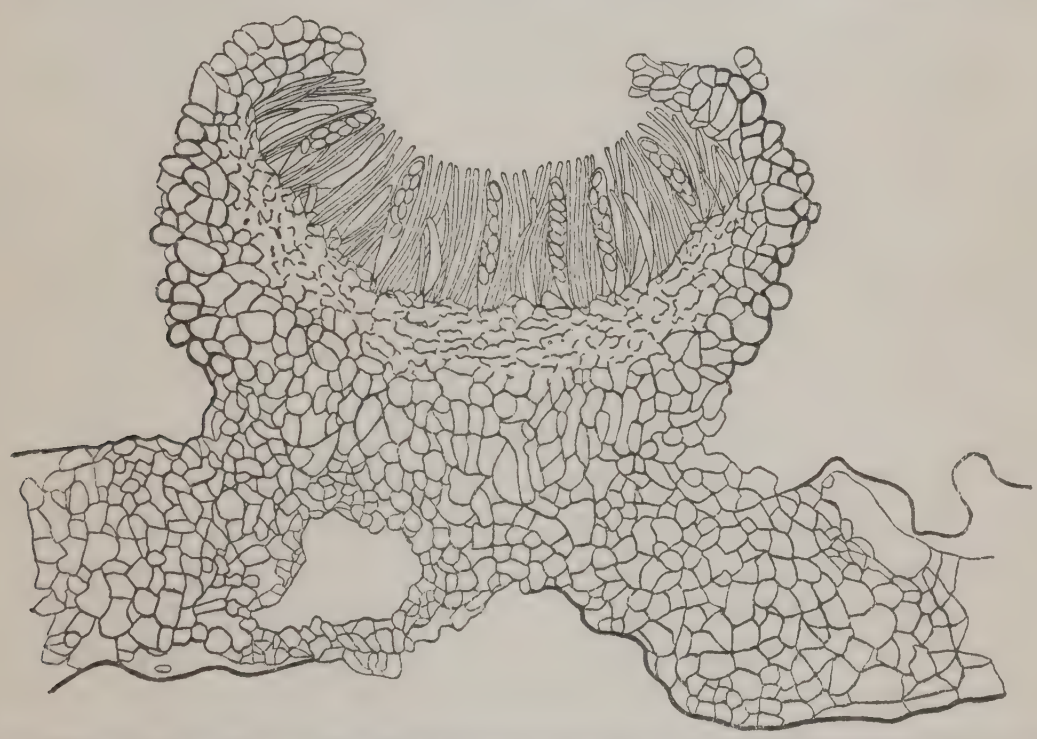

FIG. 5.—*Pyrenopeziza medicaginis:* Semidiagrammatic section of an apothecium. The tissue of the leaf has been largely replaced by the fungus hyphæ and stroma.

nutrient material added was used as a culture medium for isolation very little mycelial growth developed outside the diseased leaf fragment, but that in many cases apothecia were produced. A little less than half of the leaf fragments thus treated usually developed apothecia in about three weeks if kept at a temperature of 18 to 20° C. Above 22° apothecia occur less frequently and are not so well developed. On many of these leaf fragments conidia are not produced abundantly along with the apothecia. Thus, by selection, apothecia can be obtained which will discharge in great abundance spores free from conidia or the spores of any foreign organism. From these spores discharged on an agar surface cultures can easily be obtained. The peculiar behavior of these cultures which distinguishes them from those obtained from mycelium in the leaf tissue will be noted in the following section.

Production of Apothecia in Pure Culture

The cultures which are obtained from ascospores discharged on an agar behave in early stages of development very differently from cultures which have been obtained from mycelium in the leaf tissue. Mycelium develops very slowly. Even when the agar surface has been abundantly strewn with spores, mycelium does not become visible until after a week or 10 days. It grows almost wholly within the surface of the substratum and soon unites to form a crust that varies in color with the nutrient material furnished. Enormous numbers of conidia are produced that sometimes cover the crust with a slime. If this crust covers the entire surface of an agar slope, aerial mycelium is rarely developed in visible amount. Such a culture appears very different from one developed from mycelium from the host tissue. However, if a fragment of this crust is cut out and placed on a fresh agar surface, mycelium will emerge from its edge; and in the course of a few weeks transfers can be made which are indistinguishable from transfers from cultures obtained from host tissue.

This peculiar crust produced by mycelium from ascospores was not carefully studied until the discovery had been made that one such culture was producing apothecia. Apothecia from this and other sources were immediately used to start other cultures to determine the conditions requisite for the development of the ascigerous stage. Since the supply of ascospores was somewhat limited, and since the time required for the formation of apothecia under the best of conditions found is from five to eight weeks, it has not been possible to carry this part of the work to a satisfactory conclusion. In no case have apothecia appeared in all the cultures of a set that have been held under identical conditions. This may be due in part to the fact that it is not possible to get two slopes "seeded" with even approximately the same number of spores. Thus far the ascogenous stage has been produced in 20 cultures.

The best culture medium for this purpose appears to be oatmeal agar, though apothecia have been produced on potato agar and alfalfa stems. Spores may be discharged on a layer of clear-water agar in a petri dish, where their number and distribution may be observed before transfer with the substratum to the agar slope in the test tube; or they may be discharged directly upon the agar slope. The latter method appears to be somewhat better.

With regard to temperature and light, by far the best success has been attained in cultures exposed to weak light in a room which normally maintains a temperature of about 21° C., but which at several times during the growth of the cultures fell to 14° to 16° for several days. Continued low temperature does not appear to favor the production of apothecia. Cultures grow only slightly at 8°. Cultures held at constant temperatures below 14° have shown no indication of producing apothecia

after three months of incubation. Thus, although apothecia have been produced on artificial media in sufficient amount for inoculations and other experimental work, no method of producing them with definite certainty and in large number has been devised.

Description of Cultures on Special Media

It will be seen from the foregoing that a study of the fungus on each culture medium might properly include a comparison on that medium of cultures from three sources: (1) Ascospores discharged on that substratum, (2) transfers of mycelium developed from ascospores, and (3) transfers of mycelium obtained from plating out diseased leaf fragments. The last should be included because, inasmuch as it produces no spores that are capable of infecting the host, its identity as the pathogenic organism can only be shown by demonstrating its identity with cultures derived from ascospores which, beyond question, belong to the pathogene. Of course, it would be anticipated that the cultures from these sources behave alike, and comparisons have shown that this is the case, the only difference being that cultures from ascospores sometimes appear to have slightly greater vigor. Since cultures appear to behave somewhat differently after they have been kept on culture media a year or more, comparisons are made of only recent isolations. Such notes of the development of the fungus from ascospores will be made as appear significant.

The following descriptions are made only for the purpose of assisting in the identification of the fungus.

Cultures on Potato-Dextrose Agar (Slanted Tubes)

From transfers of mycelium.—The typical form of the culture on this substratum as developed in three or four weeks is a raised knob 3 mm. or more high, surrounded by a thinner raised growth that decreases in thickness to the edge. Four to six weeks at optimum temperature are required for the fungus colony developing from a small transfer to extend entirely across a slope in a 15-mm. test tube. At the edge of the growth the submerged mycelium is usually very slightly in advance of the aerial mycelium. The short, matlike aerial mycelium of ascending hyphæ varies greatly in color, tending to become darker at high temperatures and remaining white at low temperatures. Ordinarily it is white or gray, often with the admixture of a slightly pink tint.

Conidia can almost always be found scattered about on the aerial mycelium at various stages in the development of the culture. After the cultures are 3 or 4 weeks old, conidia are produced in more or less definite acervuli on typical conidiophores. These acervuli may be scattered about over the culture or produced in groups on a black stromatic base.

FROM ASCOSPORES.—Ascospores discharged on potato agar develop a yellow color much like that described later for oat agar, but conidia are produced early in greater abundance, and when these ooze out, the culture becomes a dirty gray. Aerial mycelium is absent except on that part of the culture which has begun to dry out.

CULTURES ON OATMEAL AGAR (SLANTED TUBES)

FROM TRANSFERS OF MYCELIUM.—Cultures on oatmeal agar resemble in form those on potato agar, except that the growth is not raised and therefore the central knob is lacking. Growth is a little less vigorous. Instead of a pink color, occasionally a yellow color is developed in a portion of the culture, which becomes matted and wet as though bacteria were present. This indicates the abundant development of conidia from the mycelium without the formation of a definite acervulus.

FROM ASCOSPORES.—Cultures from ascospores discharged on oat agar are easily conspicuous by reason of the yellow color produced, which is of much the same character as that produced on the living leaf. The intensity of the color depends in part on the number of the fungus colonies which are developed. If they are closely crowded, in the course of two or three weeks the color is ochraceous orange. If the colonies are less numerous, the color is duller, becoming a clay. Conidia are produced more abundantly from the colonies with the larger amount of surface space, being less crowded.

CULTURES ON DEXTROSE AGAR (2 PER CENT DEXTROSE AND 2 PER CENT AGAR)

FROM TRANSFERS OF MYCELIUM.—Growth is very meager. The larger part of the mycelium is submerged in the substratum, and is dark olive in color. Aerial mycelium is white. No conidia have been noted, even after a growth of six weeks.

CULTURES ON STERILE ALFALFA STEMS

FROM ASCOSPORES.—The stems used for these cultures were gathered standing through the snow in midwinter. When ascospores were discharged on these stems, a growth resembling that on oat agar was produced. In two or three weeks the color on the more moist portions of the stems was cinnamon-buff. On dry portions there was a little white mycelium. Conidia are produced in great abundance. An illustration of a way in which these conidia are occasionally borne on these stems has already been given (fig. 3). The mycelium penetrates only a few outer layers of cells of the substratum.

SPORE GERMINATION

GERMINATION OF CONIDIA

The first attempts to germinate conidia were made for the purpose of isolating the fungus. But although plates were poured repeatedly, no spore that could be identified as belonging to this fungus was ever observed to germinate.

When pure cultures had been obtained, efforts were resumed under more favorable conditions. Germination was attempted in poured plates, on an agar surface, in distilled water, and in several liquid media at temperatures within the range through which the mycelium of the fungus grows. A few doubtful cases of germination have been noted in which it could not be determined whether a structure attached to a spore was an attached conidiophore or a germ tube. But since the structure did not develop beyond the length of a normal conidiophore, it was assumed to be such.

The nearest approaches to germination have been observed in the case of spores flooded on a plate of potato agar, the culture medium upon which the fungus grows best. In early tests it appeared that a single spore sent out a lateral germ tube about 20 μ in length. Later, occasional spores on this substratum have shown a slight bulging of the cell wall, but no well-defined germination has taken place. In order to allow conidia a long time in which to develop fungus colonies, they have been flowed in a water suspension on agar slopes in test tubes. The free water was poured off and the tubes were set aside for several weeks; but no fungus colonies developed upon them.

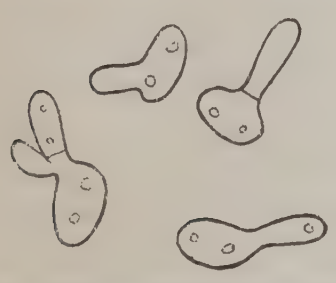

FIG. 6.—*Pyrenopeziza medicaginis:* Germinating ascospores.

Conidia upon dead leaves have been included in germination tests, but not enough of over-wintered conidia have been secured for this purpose. These results, together with the fact that no successful inoculations with conidia have been made, make it seem unlikely that they germinate often, at least not in significant numbers.

GERMINATION OF ASCOSPORES

Ascospores of *Pyrenopeziza medicaginis* germinate readily on an agar surface, or in distilled water, but very poorly, if at all, when they are submerged in poured agar plates. The percentage of germination is very variable, usually being rather low, from 25 to 30 per cent but sometimes being as high as 80 per cent. No explanation for this variability can be offered. The spore germinates from any part of its circumference with little apparent preference for the side or the end (fig. 6). The germ tube is soon cut off by a septum. Other features of germination will be discussed under temperature relations.

RELATIONS OF TEMPERATURE

GROWTH OF MYCELIUM

In order to determine the optimum temperature for the development of mycelium, transfers were made on potato agar and placed at temperatures ranging from 2° to 29° C. These were kept under observation for

six weeks. At the end of this time the growth in the cultures at the two extremes, 2° and 29°, was barely perceptible. The best growth was made at temperatures between 16° and 25°, although at the lower temperature it was somewhat retarded. Between 2° and 16° there was a remarkably regular retardation of growth in proportion to the lowered temperature.

GERMINATION OF ASCOSPORES

Three tests of the time required for the germination of ascospores at different temperatures have been made. As a result of preliminary trials, the following method of making germination tests has been found the most satisfactory. The leaf fragment or culture bearing apothecia which are discharging spores is placed in the cover of a petri dish in which a thin layer of 2 per cent water agar has been placed. Since the act of transferring the culture to the petri dish often affects the rate of discharge of ascospores for a time, at least 12 hours should be allowed to elapse before spores are taken for germination test. Then the cover of the dish is turned so that the spores fall in a new place. When a sufficient number of spores have collected (from one to three hours will be required), the area on which the spores are collected is marked with a wax pencil, and the cover is transferred to another dish of agar if more test plates are needed. The plate on which the spores have been collected is then placed at the desired temperature. The spores can easily be observed through the bottom of the plate under the low power of the microscope.

This method is open to the objection that some of the spores are exposed on the plate for one or more hours at room temperature before they are placed at the desired temperature. This would be a serious matter if the spores germinated quickly; but since nearly 12 hours are required for complete germination, it does not appear to be a large factor.

The results of the tests are compiled in Table I. At almost all of the temperatures used at least three trials have been made with spores from different cultures. The results of the trials have not been divergent outside the limits of error incidental to such tests. In Table I the numbers in the columns under the length of time during which the spores had been kept at the given temperature represent the length of the germ tube at that time in relation to the greatest diameter of the spore. This number is obtained by estimate, and not by measure. In making the estimate after the longer periods of time, the shorter germ tubes which have apparently ceased growth are disregarded, and only the length of the longer ones is considered. It may be added here that at the extremes of temperature, where germination does not proceed far, the percentage of germination is also greatly reduced.

From this table it appears that the optimum temperature for spore germination is between 12° and 26° C.

TABLE I.—*Time required for the germination of ascospores of Pyrenopeziza medicaginis*

Temperature.	Estimated length of germ tube (relative to greatest diameter of spore) at incubation periods of—				
	12 hours.	24 hours.	36 hours.	48 hours.	72 hours.
°C.					
2–3	.....	.....	.....	.....	0.5
4–5	.....	.....	.....	0.5	1
6	.....	.....	1	2	3
8	.....	1	1–2	2	3
12	.....	1–1.5	1–2	2	3
14	1	1–2	2–2.5	3–4	.....
18	1	1–2	3	4–5	5–6
21–22	1	1–1.5	.....	2–3	.....
26	1	1–2	2	3	3–4
29[a]	.....	.....	.....	.....	.....
30[a]	.....	.....	.....	.....	.....

[a] Germinating spores rare; growth not continued.

PATHOGENICITY

INOCULATIONS WITH CONIDIA

Inoculations with conidia were begun when the disease was first found in order to determine if the conidia found so abundantly in diseased areas belonged to the organism causing the disease. In no case was any degree of success in producing infection obtained. When the fungus had been cultured and had been shown to be the cause of the disease, further efforts to secure infection with conidia were made. Various methods of making inoculations were followed. Spores from diseased leaves or from pure cultures were sprayed over the plants in a water suspension. The plants were kept in a moist chamber for at least 24 hours. In order to get large numbers of spores on selected leaves, diseased leaf fragments from which conidia were oozing were placed on moistened alfalfa leaves for about 24 hours in a moist chamber. When these leaves finally became dry, the mass of conidia appeared as a slight white incrustation. This likewise failed to produce any diseased condition. In order to make conditions more severe, other leaves were slightly wounded by abrading the cuticle before inoculation was made. This likewise was unsuccessful. No conditions were found under which these conidia were viable and capable of producing infection. However, negative evidence of this kind must be kept open to question.

INOCULATIONS WITH ASCOSPORES

In contrast with the results obtained from inoculations with conidia, no inoculation with ascospores has yet failed to give a larger or smaller percentage of infection. A few of the earlier inoculations in the greenhouse in the late summer were not absolutely conclusive, because at that time all plants showed a few infections, probably from spores blown in

from an alfalfa field close beside the greenhouse; but during two winters all plants in the greenhouse except those inoculated have remained free from the disease.

Ascospores for inoculation have been obtained from dead leaves from the field, from apothecia on alfalfa leaves under conditions of pure culture, and from apothecia produced on culture media. The following method of inoculation, which was first devised to secure the discharge of ascospores from dead alfalfa leaves upon the leaf to be inoculated with the least possible danger of transferring conidia or other spores, has been used in many inoculations with this fungus. For this purpose a small glass dish is made by cementing a heavy cover glass to one side of a glass ring about 15 mm. in diameter and 6 mm. deep. In the bottom of the dish thus formed is placed a fragment of dead leaf bearing apothecia which appear mature. If dry leaves from the field are used, they should be kept thoroughly wet for at least 12 hours before being used to obtain the best discharge of spores. These leaf fragments remain firmly attached to the bottom of the glass dish when kept wet. In order to determine whether spores are actually being discharged and how abundantly, the glass dish is then inverted on a thin layer of clear-water agar in a petri dish. If, after an hour, very few or no spores are found on the agar, the fragment is discarded and a new one substituted. The agar plates are kept to ascertain whether the spores are viable.

The dishes containing material that is discharging a suitable abundance of spores is then placed over an alfalfa leaflet which is supported so that the petiole is not greatly bent. The leaf is previously wet with a fine spray, or rubbed slightly to cover it with a thin film of water, or sometimes it is sprayed after the spores are applied. After the dish has been inverted over one leaflet for an hour or more, it is transferred to another. The leaves thus inoculated are marked. It has been found that apothecia on dead leaves can usually be depended upon to maintain a discharge of spores for at least 18 hours. The inoculated plant is kept in a moist chamber for at least 12 hours after the last set of leaves has been inoculated.

Another method that has also been used consists of placing the material from which ascospores are being discharged in the top of a bell jar over plants which are well sprayed. This method has produced very abundant infection on young plants, especially on vigorous seedlings; but with the old plants infection is usually meager. Apparently, young tender leaves are much more easily infected than older ones, although infection may take place at any age.

One of these inoculations with ascospores from dead leaves made on November 4 and 5, 1915, is summarized in Table II as the result appeared on November 22. Several of the inoculated leaves had fallen at this time and are not included in the results.

TABLE II.—*Result of the inoculation of alfalfa leaves with ascospores of Pyrenopeziza medicaginis from dead leaves*

Plant No.	Conditions of inoculation.	Number of inoculated leaves.	
		Diseased.	Not infected.
1.....	Plant growing vigorously, leaves young, sprayed.....	10	11
2.....	Very vigorous plant, leaves young, rubbed till surface was uniformly wet.	6	0
3.....	Very vigorous, leaves rubbed until wet, inoculated on lower surface.	5	1
4.....	Plant not growing, leaves not young, but healthy. Surface rubbed.	8	6

Results of this kind have been obtained repeatedly with inoculations from ascospores under conditions of pure culture, as well as from dead leaves. When only very young vigorous leaves are chosen for inoculation and the material used discharges spores in abundance, a 100 per cent infection can usually be obtained. Either the upper or lower surface of the leaf can be infected.

The failure to get a 100 per cent infection in all cases appears to be due in part to the variable and often very low percentage of germination of ascospores. It has been noted especially with ascospores discharged from leaves that not more than 1 or 2 per cent of the first spores to be discharged after the leaf is soaked fail to germinate more, while 25 per cent of those discharged a few hours later may germinate. But sufficient observation of germination under these conditions has not been made to warrant the opinion that this is a general rule.

The time required for the yellow-leafblotch to appear after inoculations in the greenhouse is approximately two weeks. In case a great number of viable spores has been placed on the leaf, the yellowing begins a few days earlier; but if the number of spores is small, giving rise to but a few penetrations, the yellowing is delayed until a few days later. Heavy infection may produce yellowing prior to the development of pycnidia, and, in fact, the leaf may be killed before pycnidia can be formed. When infection is light, a leaf may not show a yellow blotch until very shortly before the pycnidia are formed. Under greenhouse conditions the diseased leaf tissue usually dries out so early that no apothecia develop. Only in a single case, where the inoculated plants were growing in a dense mat that maintained a constant condition of high humidity, did apothecia appear.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS AND CONTROL MEASURES

PRODUCTION OF CONIDIA

As has been described previously, conidia are formed in the pycnidia sometimes even before the symptoms of the disease are conspicuous. They are usually abundant by the time the yellowing is distinctly visible. It appears that they are produced in greatest amount while the leaf tissue surrounding the pycnidia is still alive. When the diseased area dies and dries, conidia production appears to cease. When the entire leaf is dead the conidiophores are usually found disorganized, though the cavity may still be filled with spores. But it is doubtful if the production of conidia always ceases with the death of the leaf. In a few instances, while examining structures that appeared like undeveloped apothecia on dead leaves in the summer; and in one instance in the spring, these structures were found to be filled with typical conidia borne on conidiophores. Since conidia production has been found under such circumstances but two or three times, it is assumed that it is not a frequent occurrence. There has been no opportunity to determine whether these conidia are capable of germination. Ordinarily by the time that apothecia are mature the conidia have completely disappeared from the diseased leaf.

PRODUCTION OF ASCOSPORES AND OVERWINTERING

Since the experimental evidence indicates that the ascospores are the only source of infection, a careful study of the conditions under which they are produced becomes important. Apothecia containing spores that will be discharged after a few hours of soaking have been found most abundantly in the autumn, and even in the early winter, especially in the late growth of alfalfa which has become well infected before killed by frost. If these leaves are not subjected to frequent wetting, spores will be retained in a viable condition, at least over the winter. This has been shown by the fact that collections made in October, 1915, and wintered in a cage out of doors were used successfully to produce infection the following February, and spores were discharged from this material as late as the following August. But in the field the spores formed in the fall appear to be discharged long before spring, and no new spores are formed until after a period of warm weather. On May 1, 1916, apothecia were collected at Madison, Wis., on overwintered leaves in the field, but no mature spores were found in them until they had been kept four or five days in a damp chamber at room temperature. On May 10 several collections of apothecia containing spores were made. By May 28 the disease was abundant in the localities where the apothecia had been found on May 10. On the latter date a few apothecia with mature

spores were found on diseased leaves of a plant in a protected location which had grown more rapidly than most plants in the open field and where infection must have occurred earlier. From this time on infection from overwintered material was reenforced with infection from ascospores from diseased leaves of the current season's growth.

During the summer, apothecia were found in great abundance in a field left uncut for seed production. During the hot weather of July and August these failed to open when soaked, and appeared to be entirely lifeless. Yet, after the crop had been cut and the rains had come and a new crop had sprung up, it was found to be very heavily infested with the leafblotch. In late October apothecia producing spores in great abundance were found in these fields, and the late autumn growth showed much of the disease.

It is of interest to note that infected leaves which remain attached to the plant appear to be much more favorably situated for the production of apothecia than those which early fall on the ground. This is true both in summer and in winter. Infected leaves which are early beaten to the ground by rains rarely develop apothecia. The overwintered leaves which produce apothecia most abundantly are those which have remained attached to standing stems until early spring.

Thus, it appears that apothecia readily survive the winter; possibly others are developed from the stroma in the leaf in spring. The cold weather of early spring appears to be unfavorable for abundant ascospore production; and the hot, dry weather of midsummer also has an inhibiting effect. So far as observation has gone, ascospores appear to be produced in sufficient amount to account for all infections that have been observed.

PENETRATION OF THE HOST

In spite of considerable effort to determine the method by which the germ tube from the germinating spores enters the leaf, only about a dozen instances of penetration have been observed. Apparently, after the germ tube has entered the leaf, the spore breaks away from the leaf easily, and the relation of the mycelium to the exterior of the leaf is hard to trace. But in the few instances which have been observed, the germ tube has penetrated directly through the cuticle either immediately beneath or close by the spore. In a majority of cases the spore has been located at the junction of two epidermal cells, but in others the spore was near the center of an epidermal cell. None of the germ tubes which have grown out over the surface of the leaf have been seen to enter. No case of entry through a stoma has been observed.

METHOD OF DISTRIBUTION

Up to the present the disease has been observed closely in but few localities; in Wisconsin it has not been seen in newly seeded fields which are several miles from old fields where the disease is known to

occur. It has been observed the first year in newly seeded fields in close proximity to old fields where the disease occurs. Thus, there is no evidence either from the study thus far made of the life history of the fungus or from field observation that indicates that the disease is carried by properly cleaned seed. But it does appear likely that the ascospores are blown at least short distances by the wind. It is also clearly evident that any infected alfalfa hay or débris might easily convey apothecia which would become a source of infection under suitable conditions.

CONTROL MEASURES

No experiments have been conducted to determine the efficiency of possible control measures. If, as now appears, the only source of infection is the ascospores, cutting the alfalfa before these are mature should greatly reduce the disease. In fact, so far as the writer has observed, this is the case. In fields which make a vigorous growth during the entire season and which are cut for hay at the stage usually recommended, this leafblotch is never important. But if for any reason an infected field is allowed to remain uncut for an unusually long period, especially in the cool, moist weather of spring and autumn, the disease becomes abundant and destructive, provided a source of infection is present. Lodged plants which have escaped cutting, or plants left standing in fence corners, may become important sources of infection at this time. Uncut plants of this character also provide excellent facilities for the overwintering of this fungus, as well as several others, and should be carefully eliminated wherever the disease is troublesome.

SUMMARY

(1) The disease of alfalfa here described as the yellow-leafblotch is one of considerable economic importance which has been recognized in America only during the last three years (1915–1917), although it has long been known in Europe.

(2) The yellow-leafblotch occurs in important alfalfa-growing regions from New Jersey to Oregon, and at least as far south as Tennessee.

(3) The injury is brought about, either directly by a slow killing of the infected leaves, or indirectly by furnishing easy access to the weakened leaves for other organisms.

(4) The causal organism (*Pyrenopeziza medicaginis*) is a fungus which produces, first, a conidial stage on the living leaves and later, ascigerous stages on the portion of the leaf which has been killed.

(5) The fungus has been grown in culture, where both the conidial and ascigerous stages have been produced.

(6) Infection appears to take place only from ascospores, which upon germination are able to penetrate the epidermal cells of the leaf. The viability of the conidia has not been conclusively demonstrated.

(7) The fungus overwinters on dead leaves which were infected the previous autumn.

(8) Cutting infested fields before the ascigerous stage of the fungus has developed on infected leaves appears to hold the disease in check.

(9) Control measures when necessary must apparently be developed as a method of sanitation that will remove the dead leaves on which the apothecia develop.

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PLATE D

Alfalfa showing the yellow-leafblotch.

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PLATE 25

Pyrenopeziza medicaginis: Conidial (Sporonema) stage on a leaflet of alfalfa.

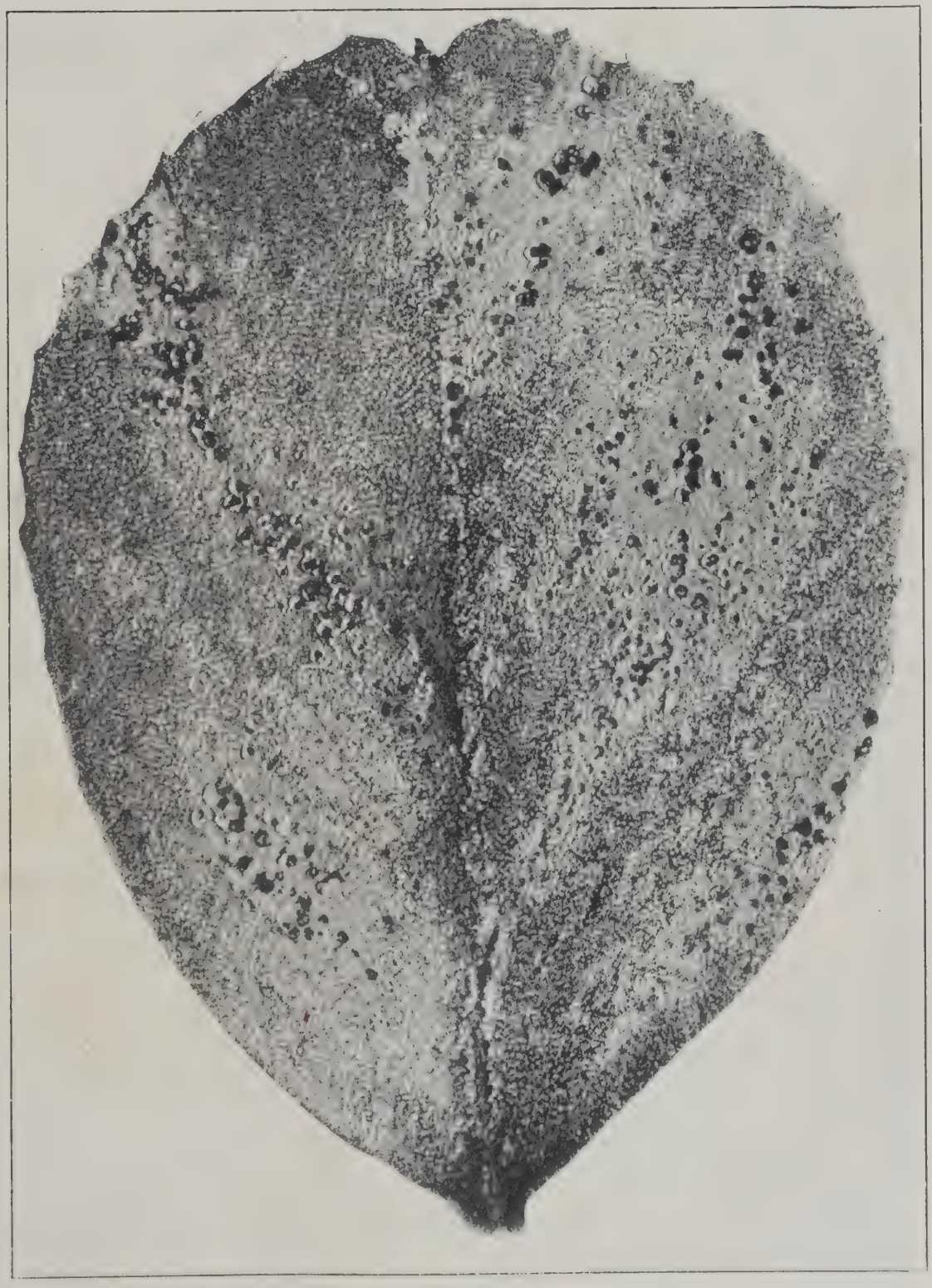

PLATE 26

Pyrenopeziza medicaginis: Apothecia on the lower surface of a dead leaflet of alfalfa.

AN UNDESCRIBED CANKER OF POPLARS AND WILLOWS CAUSED BY CYTOSPORA CHRYSOSPERMA

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INTRODUCTION

In the semiarid regions of the Southwest tree growth is not favored by climatic conditions as it is in the East. This is especially true of ornamental and shade trees suitable for homes, streets, and parks. Several species of poplar (*Populus* spp.) and willow (*Salix* spp.) are able to grow into beautiful ornamental and shade trees under the adverse conditions in many of the Western States. Since there are so few species of shade trees capable of growing in semiarid regions, it is of great importance that special attention be given to any disease which may attack these trees.

From time to time specimens of diseased bark from various species of poplar have been received by the writer from several Western States, accompanied by statements that the trees were being killed and requests for information as to the cause of the trouble. On account of the seriousness and wide distribution of the disease, investigations as to its cause and control were undertaken. This article gives the results of these investigations.

The disease has been found at various altitudes, ranging from 1,000 to 8,000 feet. It is common in the semiarid regions of the Southwest on various species of poplar, especially when used as shade or ornamental trees, and occasionally on willows.

DESCRIPTION OF THE DISEASE

This disease occurs in the form of lesions or cankers on the trunks and large limbs of affected trees. It is also found attacking the small branches and twigs. These are usually killed without forming any definite canker.

The lesions caused by this disease resemble what is often called "sunscald" on the trunks of fruit trees. The diseased bark is gradually killed in more or less circular areas. Young infections on smooth-barked shoots can be recognized by the presence of brownish, shrunken patches. However, these differ but little in general appearance from the surrounding healthy bark.

The area invaded by the fungus may be fairly regular or very irregular in outline. The diseased area gradually enlarges until the trunk or

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branch is entirely girdled and killed. The fungus often enters the dead tips of twigs or small branches. It then gradually kills the invaded branches back to their juncture with a larger branch or with the trunk. The fungus then develops on the large branch or the trunk a more or less circular canker around the base of the dead twig. Reddish fruiting pustules may appear on the dead areas near the edge of the canker; or in smaller branches they may appear over the entire surface killed by the fungus. The inner bark of the diseased areas gradually turns black and often gives off a foul, salty odor. The sapwood, especially the medullary rays, is also diseased and is stained a watery, reddish brown (Pl. 27, A), and the heartwood is sometimes discolored. Trees 3 to 6 inches in diameter which are severely attacked have but a few, sickly looking leaves (Pl. 27, B), and such trees usually die in two or three years. Trees which have been rapidly girdled by this disease often develop sprouts from the roots (Pl. 27, C) in a manner similar to that of the chestnut when attacked by the chestnut-blight fungus (*Endothia parasitica*). These suckers are usually ultimately killed by the invasion of the fungus from the old diseased parent stem.

Canker often attacks the old trunks and large branches of various species of poplar. In such cases the fungus causes but little, if any, perceptible change in the outward appearance of the bark. The attacked area is ultimately killed, and the typical dark-red spore horns of the fungus which causes this disease are developed in the fissures of the bark.

The silver-leaf poplar (*Populus alba*) when attacked by this disease dies branch by branch, since the disease usually enters the tips of the branches in the top and gradually works downward. The cankers found on the branches of this species usually extend from one to several feet farther on the under side of the branch than on the upper side. When the canker reaches the trunk of the silver-leaf poplar, it usually travels more rapidly longitudinally than transversely. This results in long, narrow, dead areas extending often for several feet down the tree. Finally the upper portion of the crown is killed, just as the individual branches were. When a tree is slowly killed from the top downward, few, if any, suckers are developed from the roots.

The dead areas produced on trees by this disease finally develop characteristic spore horns consisting of irregularly twisted threads (Pl. 27, D), which are often flattened, ranging in color from grenadine red[1] to English red. The spore horns when first formed are soft and sticky, but they dry rapidly and become hard and brittle.

On the young branches and small trees of the aspen (*Populus tremuloides*) the typical lesions or cankers of this disease occur, but on the large, old aspens another type of canker is common in the mountains of Arizona and New Mexico. These cankers are perennial and of much

[1] Ridgway, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C., 1912.

slower growth. They usually originate from some wound in the trunk or from a dead branch. The disease first forms a circular dead area on the trunk around the base of the dead branch or around the wound through which it entered. The tree then attempts to limit the disease by developing a ring of callus around the infected area. The mycelium of the fungus, however, gradually grows under this callus and kills a new zone of tissue. This process is repeated year after year until there is formed a large canker consisting of successive rings of dead tissue. The old dead bark finally separates from the sapwood, leaving the dead area more or less exposed. The surface of this dead sapwood shows the concentric rings of dead callus which formed annually in the attempt to check the disease. No fruiting bodies of any kind have been found associated with these lesions, and it is very doubtful if this peculiar type of canker is caused by the disease discussed in this article.

Willows have also been found attacked by this canker disease, especially the weeping willow (*Salix babylonica*), which is often planted as an ornamental and shade tree. The willows are killed in much the same manner as the poplars

THE FUNGUS

This canker of poplars and willows is caused by a definite species of fungus which grows as a parasite in the bark and to a limited extent in the sapwood of infected trees. The causative organism is *Cytospora chrysosperma* (Pers.) Fr., since inoculations of this fungus made into healthy poplars have produced the typical cankers, and pure cultures of this organism have been reisolated from the cankers thus produced.

After the mycelium of the fungus has been growing for several weeks in the bark, it forms fruiting pustules or pycnidia. These pycnidia produce a large number of curved, hyalin, 1-celled spores which are extruded from the pycnidium in the form of threadlike irregular coils. These are called "spore horns" or "tendrils" (Pl. 27, D). The development of the spore horns is not limited to any special season, but may occur during any month of the year. The production of pycnidia seems to be limited to the bark, at least in the semiarid regions of the western United States, since no evidence of fruiting bodies of the fungus has been found on decorticated limbs, trunks, or stumps of affected trees.

The pycnidia are not evident on the surface of the bark until after the spore horns have been dissolved and washed away by rains; then the dead bark shrinks and the mouths of the pycnidia become evident (Pl. 28, A). Only the pycnidial stage of this fungus is known.

INVESTIGATIONS AS TO THE CAUSE OF THE CANKERS

Pure cultures of *Cytospora chrysosperma* were made by means of single spore colonies from spore horns obtained from *Populus wislizeni* and *P. alba*. Inoculations were made on small bushes of *P. alba* with material

from two sources: (1) Spores from spore horns and (2) spores from pure cultures. The typical lesions of this disease were produced by both methods.

Three series of inoculations were made under control conditions in the laboratory at Albuquerque, N. Mex., as follows: September 7, 1915, September 22, 1916, and December 21, 1916. In each series 12 plants were inoculated with the spores of *Cytospora chrysosperma*, and 6 plants were used as controls. In the first and third series all of the plants inoculated with the fungus developed the typical lesions of this disease. In the second series 9 out of the 12 plants were infected, but all of the control plants in each of the three series remained healthy. All inoculations were made by incisions through the bark (about ⅔ inch long) with a sharp, sterile scalpel. The spore horns were first dissolved in sterilized water, and this water was introduced into the incisions. In inoculations from pure cultures, the spores used came from soft spore horns grown on the surface of petri dishes. These spores were not put into water, but a small quantity of the spore mass was introduced with a sterile scalpel into the incision. Incisions which were not inoculated with the spores were made as controls. All incisions, including the controls, were immediately wrapped with wet absorbent cotton, which was left on the inoculated plants for 10 days. All the plants were kept in the laboratory and watered at the ground surface. The stems and tops of each plant therefore never had any water on them after being inoculated. By this method all chances of outside contamination were practically eliminated.

In 10 to 15 days after the plants had been inoculated, typical lesions of this disease began to develop in the shape of shrunken, dying areas about 4 mm. wide at the points of inoculation, but the control plants were healing normally. These lesions gradually spread until the stem was finally girdled at the point of inoculation, and the upper part killed. The first evidence of infection was usually a shrinking of the bark on the diseased area; later, on very young twigs the diseased bark turned black. Stems 6 to 12 mm. in diameter at the point of inoculation were entirely girdled in from two to four months. In some instances sprouts were developed below the girdled areas (Pl. 28, B) on the inoculated plants. In nature trees 1 to 3 cm. in diameter have been found which had been entirely girdled and killed by *Cytospora chrysosperma* in one year. On account of the small size (6 to 12 mm. in diameter) of the plants used in the laboratory for inoculating experiments and the dryness of the air indoors, the plants inoculated and killed by the fungus did not form any spore horns. The fungus was reisolated, however, from the cankers by taking small pieces of the inner bark at the boundary between the sound and diseased areas and placing these pieces in artificial culture media. Five or six weeks after the media had been inoculated with the diseased bark, the typical spore horns of *C. chrysosperma* began to develop in the tubes and petri dishes.

CULTURAL STUDIES

MEDIA.—*Cytospora chrysosperma* has been grown by the writer on two culture media: Corn-meal and malt agars. This fungus will probably grow on any of the usual media, to judge from the growth made on these two.

ISOLATION.—The fungus is easily isolated by removing under sterile conditions a small piece of the diseased tissue of the inner bark and transferring it to agar tubes. It can also be isolated by means of the pycnospores on agar slants or by the poured-plate method.

PHYSIOLOGICAL CHARACTERS

Certain characters are common to the growth of this fungus on corn-meal and malt agars. The most characteristic reactions were obtained with pure pycnospore cultures on 2 per cent malt agar, +7 (Fuller's scale).

On this medium the following characters develop when streak cultures are made:

The mycelium begins along the streak as a white cottony growth which spreads rapidly toward the sides of the tube. In seven days at ordinary room temperature the white color of the aerial mycelium gradually changes to a light buff, while in 10 days the submerged mycelium seen in mass is turning black. In 16 days the aerial mycelium is cottony in character and covers the entire exposed surface of the agar slant. It now varies from light buff to warm buff in the upper portion of the slant, while the lower portion is turning to a neutral gray and is becoming matted and adherent to the surface of the agar. The submerged mycelium is now black in mass and shows as a very distinct and characteristic border along the edge of the agar. Twenty days after the tubes had been inoculated, very small pycnidia, 0.4 to 1 mm. in diameter, were developed at the edge of the cultures next to the glass in the petri dishes and test tubes which were exposed to strong, diffused light. These small pycnidia contained typical pycnospores, which were discharged against the glass sides of the tubes in place of into the air. The test tubes of malt agar 20 to 25 days after inoculation show a very characteristic color reaction when the tubes are viewed from the rear of the slant—viz, the agar has retained its normal color except at the edge of the agar and air; here is seen a black layer of submerged mycelium bordered above by a layer of white to warm-buff aerial mycelium.

As the cultures in the tubes grow older, much of the aerial mycelium gradually becomes wet, mats together, and adheres to the surface of the agar, varying from mouse-gray to black in color; here and there elevated patches of white to light-buff mycelium remain (Pl. 28, C). These elevations are 3 to 6 mm. in diameter, hemispherical in shape, and contain the pycnidia. About 30 days after inoculation large typical pycnidia mature and begin discharging spores (Pl. 28, C). On plate cultures of malt agar this fungus has the same general characters of growth, except that the

aerial mycelium after about three weeks has changed to a mouse-gray color in the center of the plate, but at the margin it still retains its white to light-buff color. The entire surface of the plate is now covered with many elevated pustules 4 to 7 mm. in diameter. These develop pycnidia later if sufficient moisture is available. These pycnidia extrude a mass of pycnospores which is orange-chrome when fresh and soft, but which changes to English red on drying and hardening. These spore masses rarely form long spore horns, but usually remain as large orange-chrome drops at the point of issuance. However, if the air in the plate is dry, the typical spore horns of *Cytospora chrysosperma* are developed. Only a few pycnidia mature and discharge their spores at a time. This gradual ripening of pycnidia and the subsequent discharge of the spore masses may continue for two or three months after the tube or plate is inoculated, the length of time depending upon the quantity of culture medium present and the rapidity with which it dries.

Pure cultures of this fungus on 2 per cent corn-meal agar, +0.25, are very similar to those on malt agar, except that the mycelium produced, both aerial and submerged, is much less in quantity, and no pycnidia, or only a few, are finally developed.

MORPHOLOGICAL CHARACTERS

The hyphæ of the aerial mycelium are hyalin, fairly uniform in size, ranging from 2 to 4 μ in diameter, and very sparingly branched, with septa few and distant; the submerged mycelium is dark, neutral gray to blackish slate, black when seen in mass. Often several of these hyphæ are joined into a long bundle from which individual hyphæ put off at intervals. Individual submerged hyphæ are 2 to 4 μ in diameter, sparingly branched, and distantly septate. The black submerged mycelium does not penetrate deeply into the agar, but is more or less limited to the substratum immediately beneath the aerial growth.

DISSEMINATION OF THE DISEASE

The canker, as previously stated, enters the host through wounds or dead twigs and branches. Once established in the growing inner bark, it is easy to see how other parts of the tree are infected. During every rainy or damp spell some of these spore horns which have formed on the diseased area are dissolved, and the water containing the spores runs down the tree, in this manner transmitting them to wounds or dead twigs present on the tree, and thus originating new lesions.

At present only pycnospores are known, and since these are borne in gelatinous threadlike horns, it is impossible to state definitely with our present knowledge of this fungus how it travels from tree to tree. It is probable, however, that large numbers of these spores, after once being freed from the spore horns by rains, dry and are carried by the wind to other trees. Many spores are washed into the soil at the base of the

infected trees, and the high winds common in the semiarid regions of the Southwest could easily lift into the air the spore-laden particles of dirt and carry them long distances, thereby infecting other trees. Birds and insects may also play a minor rôle in carrying the pycnospores. In the towns and cities of the Southwest where this disease is most prevalent, the only bird present to any extent is the English sparrow.

The shipment of nursery stock infected with this disease may explain its presence in many isolated places where there is no natural growth of its host to normally harbor and transmit the disease. An instance of this kind was seen at a home in the plains country in eastern New Mexico. Two species of poplar (*Populus deltoides* and *P. italica*) had been planted from two different nurseries. Sixty-five per cent of the young trees obtained from one of these nurseries were either dead or were seriously attacked by the disease within four years, and the trees from the second nursery were in good condition, except here and there a tree was being infected from the diseased material from the other nursery. In this instance the disease was apparently introduced in the nursery stock.

CONDITIONS UNDER WHICH CYTOSPORA CHRYSOSPERMA BECOMES A SERIOUS PARASITE

There are four general conditions under which this disease usually does much damage in the Southwest: (1) On trees which are growing at the outer limits of their range and are therefore in more or less unfavorable environment; (2) On trees planted in streets, lawns, and cemeteries, where they have been weakened from neglect and lack of sufficient water; (3) On trees which have been severely pruned, as in pollarding; (4) On cuttings in propagating beds, where the usual method of propagation is used.

When poplars are growing at the extreme limits of their range or are planted in the treeless regions of semiarid countries, *Cytospora chrysosperma* becomes a serious parasite. The aspen at the lower limits of its range is often attacked, and the smaller trees are killed outright by this disease, while the larger trees are sometimes seriously injured. The instance given under the dissemination of this disease through nursery stock in eastern New Mexico (p. 337) where 65 per cent of the trees were killed shows how virulent this fungus can become when once established on plants growing in treeless regions. The following is another instance of the same kind from western Kansas.

Near Syracuse, in Hamilton County, many of the Carolina poplar trees (*Populus deltoides*) planted as shade trees are being killed by this disease.[1] Syracuse is located in the western portion of Kansas where trees are very scarce and where the rainfall is very light. In such a region trees are

[1] The data concerning the presence of this disease at Syracuse were kindly furnished by Prof. L. E. Melchers, of the Kansas Agricultural College Experiment Station.

considered a great luxury, and any person who succeeds in growing them always takes the greatest care not to lose them. The disease seems to be very virulent and is reported to be killing practically all of the Carolina poplars in that region. This illustrates how serious this disease becomes on species of poplar which are growing under unfavorable conditions.

The canker seems to be widely distributed in North Dakota and is causing serious damage to the poplar groves of that State, according to the following extract from a letter to the writer from State Forester Fred W. Smith:

> The poplar groves in this State are pretty largely infected with *Cytospora chrysosperma*. This disease seems to be affecting all of the poplars with the exception of the native poplars, *P. balsamifera* and *P. tremuloides* var. *candicans*. The common poplars distributed by the nurseries seem to have been all badly infected. Tens of thousands of trees have been killed in North Dakota this last year by this disease.

Poplars, when planted in dry climates on streets, lawns, and in cemeteries, are often very subject to the attacks of *Cytospora chrysosperma*, especially when the young trees are not watered regularly and abundantly. When the trees do not receive enough water, the twigs and small branches in the top gradually die. Through these dead and dying branches the fungus readily enters. This is especially true of certain species of poplar which are highly susceptible to the disease. Hundreds of shade trees in Arizona, New Mexico, and Texas have been seen by the writer which were either dying or had been seriously injured by this fungus. Most of these trees had been much neglected and undoubtedly had not had a sufficient amount of water.

In many of the towns of Arizona and New Mexico the habit of pruning large living limbs, and, in some cases, of pollarding the entire tree, is common. When large limbs are cut off in this dry climate, the exposed ends of the portion left on the tree die back from drying some 3 to 12 inches. The tree may then put out new branches below the dead portion. Later, this young growth is often killed by the gradual advance of this canker which entered at the exposed dead surface of the severed branches.

The writer has received specimens of Carolina poplar cuttings taken from propagation beds attacked by this fungus. The method usually followed in propagating trees of the genus Populus in nurseries is to take cuttings about 8 to 10 inches long and 0.25 to 0.5 inch in diameter and place them in the ground with the upper part projecting some 2 to 3 inches. Such propagation stock is often seriously damaged by *Cytospora chrysosperma*. This fungus attacks the exposed ends of the cuttings and often kills them before any shoots appear, or the cuttings may put out sprouts which are later killed by the fungus which gradually travels down the cutting into the bark below the young shoot, thereby girdling the stem and shutting off the food supply from the sprout (Pl. 28, D).

How serious this disease may be when it attacks poplar cuttings in the propagating beds is shown by the following data[1] from the Fort Hays Experiment Station nurseries situated at Hays, Kans. In 1915 two different plots containing about 10,000 Carolina poplar cuttings each were planted. On one of these plots situated on level creek-bottom land about 95 per cent of the cuttings started to grow. When the larger ones were about 10 inches tall, they began to turn yellow, quit growing, and gradually died until there was less than 1 per cent alive at the close of the season, and these were only 1 to 2 feet high. The second block of cuttings was located about 20 rods from the other block. These cuttings started nearly as well as those in block 1, but gradually died in the same manner until only about 40 per cent were alive at the end of the season. Most of the plants in this second bed grew about 3 feet high, a few were 4 to 5 feet tall, and many were only 1 to 2 feet in height. Specimens of the diseased cuttings from these two plots examined by the writer had the typical red spore horns of *Cytospora chrysosperma* which had entered at the exposed cut ends of the cuttings and had finally killed them.

The remedy for this loss in cuttings is to change the system of propagation for those species of poplar which are susceptible to the disease. The writer has seen the following method of propagating the Carolina and Lombardy poplars used with good success in the dry regions of New Mexico. The small branches or twigs which have been selected for propagating purposes, instead of being cut into 6- or 8-inch pieces, are placed entire in the bottom of a trench 2 to 4 inches deep. They are then entirely covered with sand, kept damp by frequent watering, but well drained; and in due season the dormant buds along the twigs grow and send up shoots, while an abundance of roots develop along the buried twig. Propagating stock thus handled does not present any exposed surface to be infected by the fungus.

DISTRIBUTION OF THE FUNGUS

Cytospora chrysosperma is rather widely distributed in certain sections of the United States, especially in the Southwestern States. It ranges from Texas and Kansas northward to Montana and westward to California. It has been found in nine States—Arizona, Colorado, Kansas, Montana, Nevada, New Mexico, North Dakota, South Dakota, and Texas; and also in Mexico. The fungus is widely distributed in Europe, having been reported from Germany, Austria, Switzerland, Italy, France, and Sweden.

[1] The above data were obtained through the kindness of Mr. J. W. Preston, formerly in charge of the Fort Hays Experiment Station.

DISTRIBUTION IN THE UNITED STATES

Cytospora chrysosperma has been reported from and collected[1] in the following places in the United States:

On *Populus* sp. (Alamo poplar):

El Paso, Texas, August, 1915.

On *Populus acuminata:*

Cascade, Montana, by WILLIAM LOCHRAY, August, 1913 (FP 20705).[2]

Albuquerque, May, 1916; **Chester,** November, 1916 (FP 21716); and **Deming,** New Mexico, November, 1916 (FP 21030).

Brookings, by H. F. COE, August, 1913 (FP 15799), and **Spearfish,** South Dakota, by H. F. Coe, September, 1913 (FP 15975).

On *Populus alba*:

Albuquerque, New Mexico, September, 1915, and July, 1916 (FP 19515, 19621, and 21250).

El Paso, Texas, August, 1915.

On *Populus angustifolia:*

Flagstaff, Arizona, July, 1915.

Denver, Colorado, by E. BETHEL, June, 1913 (FP 8460).

Roswell, November, 1916, and **San Mateo Mountains**, New Mexico, August, 1915 (FP 19586).

On *Populus balsamifera-suaveolens:*

Ulander, North Dakota, by B. T. GALLOWAY, September, 1916 (FP 21508).

On *Populus deltoides:*

Flagstaff, Arizona, July, 1913 (FP 19510).

Hays, by J. W. PRESTON, December, 1915 (FP 21029), and **Syracuse**, Kansas, by L. E. MELCHERS, 1915.

Golconda, Nevada, 1908 (FP 1260).

Albuquerque, August, 1915, and January, 1917 (FP 19500 and 21766); **Capitan,** by J. W. O'BYRNE, July, 1917 (FP 2937); **Deming,** December, 1915; **Endee**, by A. S. REEVES, April, 1915 (FP 19503), and May, 1915; and **Socorro,** New Mexico, July, 1915 (FP 19587).

El Paso, August, 1915; and **San Marcos,** Texas, November, 1915 (FP 19726).

On *Populus italica:*

Flagstaff, Arizona, July, 1915 (FP 19448).

Albuquerque, throughout the entire year 1916; by P. W. SEAY, December, 1916 (FP 21762 and 21765); **Isleta**, New Mexico, November, 1916 (FP 21588).

El Paso, Texas, August, 1915.

On *Populus macdougali:*

Phoenix, December, 1915; **Tucson,** December, 1915; and **Yuma,** Arizona, December, 1915 (FP 21026); **Deming,** New Mexico, December, 1915 (FP 21031).

On *Populus sargentii:*

Denver, Colorado, by E. BETHEL, June, 1913 (FP 8431).

On *Populus tremuloides:*

Flagstaff, Arizona, July, 1915 (FP 19447).

Cienega Ranger Station, 1914 (FP 19622); **Cloudcroft,** August, 1915 (FP 19623); and **Tejano Experiment Station,** New Mexico, by P. W. SEAY, December, 1916 (FP 21718).

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[1] All of the collections cited were made by the writer unless otherwise stated.

[2] "FP"=Forest Pathology Investigations.

On *Populus wislizeni:*

Flagstaff, Arizona, July, 1916 (FP 21262).

Albuquerque, November, 1915 (FP 19919); by R. M. HARSCH, September, 1916 (FP 21605); and **Domingo,** New Mexico, by R. M. HARSCH, August, 1915.

El Paso, August, 1915; and **Pecos,** Texas, November, 1916 (FP 21717).

On *Salix amygaloides:*

Denver, Colorado, by E. BETHEL, June, 1913 (FP 8432).

On *Salix babylonica:*

Albuquerque, New Mexico, September and August, 1915 (FP 19493).

On *Salix wrightii:*

Yuma, Arizona, December, 1915 (FP 21764).

DISTRIBUTION IN MEXICO

On *Populus* sp. (Alamo poplar), **Juarez,** 1912.

On *Populus italica,* **Juarez,** 1912.

On *Populus wislizeni,* **Juarez,** 1912.

From the foregoing data it will be noted that 14 species of trees are attacked by *Cytospora chrysosperma*, as follows: *Populus acuminata*, *P. alba*, *P. angustifolia*, *P. balsamifera-suaveolens*, *P. deltoides*, *P. italica*, *P. macdougali*, *P. sargentii*, *P. tremuloides*, *P. wislizeni*, *Populus* sp. (Alamo poplar), *Salix amygdaloides*, *S. babylonica*, and *S. wrightii.*

CONTROL OF THE DISEASE

Certain species of poplars have been found to be more susceptible to this disease than others. Therefore, only those species of poplars which are most resistant to the disease should be selected for planting in regions where this disease is common. The Carolina poplar and the silver-leaf poplar are highly susceptible to this disease under the conditions obtaining in a dry climate like that of the Southwest. When these two species of trees are about 12 to 14 inches in diameter at the ground, they usually show, in the crown, an increasing number of large, dead branches which have been killed by *Cytospora chrysosperma.* In fact, the writer has never examined a large tree of either of these two species in Arizona or New Mexico that has not been seriously injured or finally killed by the disease. This is particularly true in the vicinity of Albuquerque, N. Mex. The Carolina poplar is also attacked in this western country by two insect parasites which weaken the trees and make them unsightly and more easily infected by this disease. One is the cottony scale (*Pulvinaria* sp.), which usually seriously deforms the trees, and the other insect is the poplar borer (*Saperda* sp.). This species of poplar has, therefore, three serious enemies with which to contend in this arid country and should not be planted as an ornamental or shade tree.

The native valley cottonwood (*Populus wislizeni*) is highly resistant to the disease caused by *Cytospora chrysosperma* when given any kind of care and attention and should be selected for planting in those portions of the Southwest within its range. On account of the large amount

of cotton produced by the pistillate trees of the valley cottonwood, only the staminate or non-cotton-bearing trees should be planted.

The Lombardy poplar is also resistant to this disease, but not as much so as the valley cottonwood.

Cytospora chrysosperma is not an active vigorous parasite on the well-cared-for, more resistant species of poplar. It therefore follows that in controlling this disease the trees should be given plenty of water, and care should be taken not to injure the bark of the tree in any way; particularly should the cutting off of large branches be avoided. In general, the most resistant species for planting should be selected, the trees should be given a sufficient amount of water, and should be protected against injury from lawn mowers, horses, etc. Trees thus selected and taken care of will be practically immune to the disease.

Since this disease is known to be a serious parasite in nurseries and propagating beds and to be distributed by means of such diseased stock, it is of the utmost importance that all nurseries which supply poplar stock to the dry regions of the western United States should be inspected for this and other serious diseases of poplars like *Dothichiza populea*.[1] All suspicious as well as plainly diseased stock should be destroyed by burning. General precautions of this nature, if taken, will do much to control the introduction of this disease into new territories.

If a tree already has this disease, it may often be saved by cutting off the infected branches at least 12 inches below where any signs of the disease can be detected. The ends of the branch should be painted with creosote or coal tar. If the tree is small and has a large canker on the main stem involving more than one-third of the circumference of the tree at that point, it would be best either to plant another tree or to cut the tree back to the ground and let one of the most vigorous suckers or sprouts grow. It would be impossible to cut out all of the diseased tissue on the trunk of a small tree without practically girdling it.

If the tree is 12 inches or more in diameter where infected, and the lesion is small, the disease may possibly be eradicated by cutting out all of the diseased bark and the discolored sapwood. A layer of sound bark 2 inches wide should then be cut from around the diseased area with a thoroughly sterilized knife. All of the surface exposed by the pruning operation should be painted with a strong solution of shellac or coal tar to prevent reinfection.

SUMMARY

(1) A serious canker of poplars and willows is prevalent throughout the semiarid regions of the southwestern United States.

(2) This disease is caused by *Cytospora chrysosperma*.

[1] Hedgcock, G. G., and Hunt, N. R. Dothichiza populea in the United States. *In* Mycologia, v. 8, no. 6, 300–308, pl. 194–195, 1916. Literature cited, p. 308.

(3) Pure cultures of this fungus were isolated from the diseased areas, and the typical lesions of the disease were produced by inoculating healthy poplar plants with pure cultures of the fungus. The fungus was reisolated from the cankers produced by the inoculations.

(4) Pure cultures of *C. chrysosperma* on malt and corn-meal agars were made, and the cultural characters of the fungus determined.

(5) The fungus enters the host through wounds and dead branches.

(6) *C. chrysosperma* is a serious parasite on poplars in the Southwest under the following conditions: (a) On trees which are growing at the outer limits of their range and are therefore in a more or less unfavorable environment; (b) on trees planted in streets, lawns, and cemeteries where they have been weakened from neglect and lack of sufficient water; (c) on trees which have been severely pruned, as in pollarding; (d) on cuttings in propagating beds where the usual method of propagation is used.

(7) The fungus *C. chrysosperma* occurs in nine States and on 14 different species of trees. It is also found in Mexico and in Europe.

(8) The best method of controlling this disease is as follows: (a) The most resistant species should be selected; the trees should be given an abundance of water, and should be protected against mechanical injuries; (b) a strict supervision should be established over all nurseries handling poplar stock intended for distribution in the semiarid regions of the western United States; (c) all nursery stock which shows the slightest indication of the disease should be destroyed.

PLATE 27

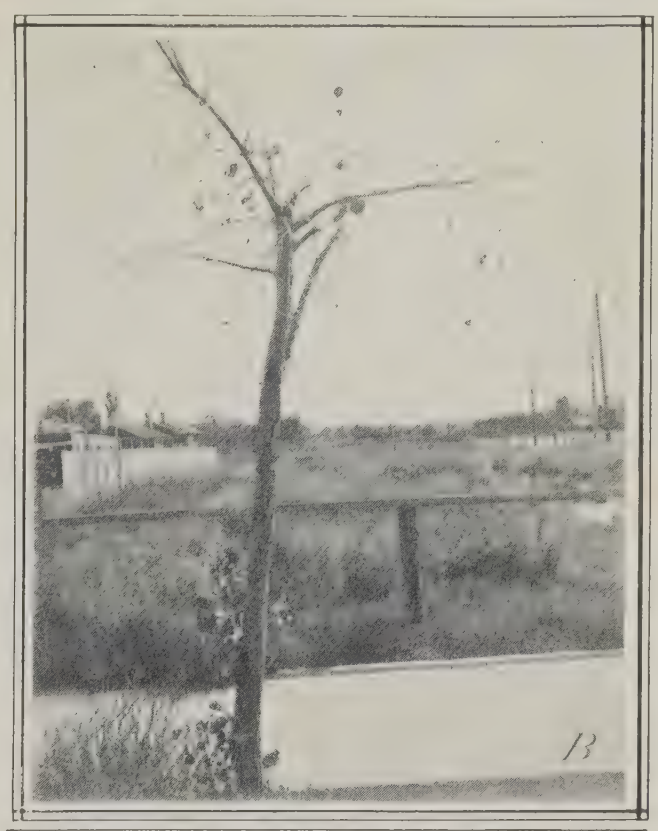
A.—A small canker caused by *Cytospora chrysosperma* on the trunk of a tree of *Populus italica*, with the bark cut from around canker.

B.—A tree of *Populus wislizeni* on the streets of Albuquerque, N. Mex., dying from the attacks of *C. chrysosperma*.

C.—Main stem of a young tree of *Populus italica* killed by *C. chrysosperma*, showing young sprouts at the base of the tree.

D.—A branch of *Populus wislizeni* attacked by C. *chrysosperma*, showing the spore horns of the fungus.

A


B

C

D

A

B

C

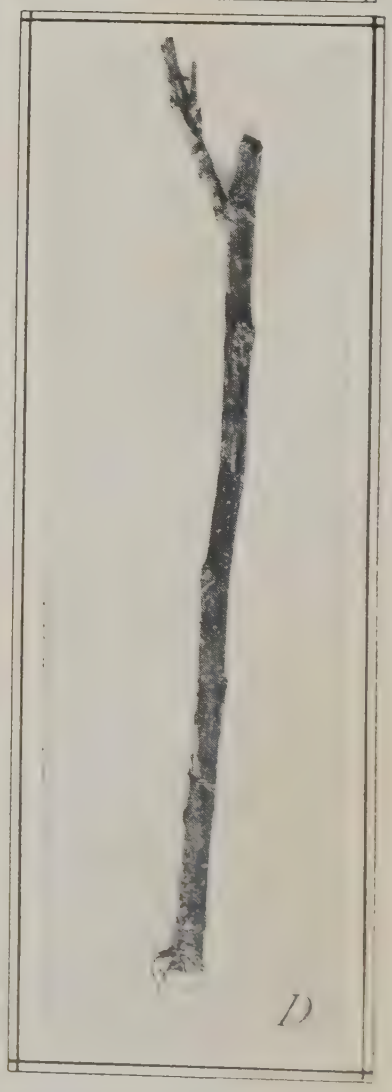

D

PLATE 28

A.—Pycnidia of *Cytospora chrysosperma* on *Populus alba* after the spore horns have been washed away by rains.

B.—A young plant of *Populus italica*, showing the upper portion of the stem killed by inoculation with *C. chrysosperma*. Sprouts are putting out below the point of inoculation.

C.—Two tubes of pure cultures of *C. chrysosperma* on malt agar, showing pycnidia and spore droplets.

D.—A propagation cutting of *Populus deltoides* from Hays, Kans., killed by *C. chrysosperma*.

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CHEMISTRY OF THE COTTON PLANT, WITH SPECIAL REFERENCE TO UPLAND COTTON[1]

By ARNO VIEHOEVER, *Pharmacognosy Laboratory*, and LEWIS H. CHERNOFF and CARL O. JOHNS, *Protein Investigation Laboratory, Bureau of Chemistry, United States Department of Agriculture*

THE PROBLEM

The main purpose of the investigation reported in this paper was to isolate the substance which proves so attractive to the boll weevil, an attraction causing such disastrous losses to the cotton industry.[2] While this paper chiefly concerns the isolation of the glucosids and their products of hydrolysis, preliminary studies of an ethereal oil which has been isolated from different parts of the cotton plant are also discussed. This oil has been found decidedly attractive to the boll weevil.

It was deemed important, furthermore, to ascertain whether or not cotton (*Gossypium* spp.) grown in this country, and especially the Upland species (*Gossypium hirsutum*), contained any substances isolated previously by Perkin from Indian or Egyptian types. Since results of the writers brought out the fact that Upland cotton was different in chemical composition from any of those previously investigated by Perkin, it was considered advisable to report briefly his work on the chemistry of other types of cotton. These investigations, it is hoped, will emphasize the importance of establishing definitely the type of cotton used in any further work.

I.—THE GLUCOSIDS AND THEIR PRODUCTS OF HYDROLYSIS

During the course of his investigations Perkin (4)[3] found that the white flowers of Indian cotton (*G. neglectum* var. *roseum*)[4] were devoid of dyeing properties, and the pink flowers of *G. sanguineum* contained only traces of a coloring substance, probably quercetin. The flowers of Egyptian cotton, the yellow flowers of common Indian cotton (*G. herbaceum*), as well as the yellow flowers obtained from another Indian cotton (*G. neglectum*) contained isoquercitrin and gossypitrin. Usually quercetin and gossypetin could be found in the extracts as products of hydrolysis of these glucosids. No gossypitrin could be found in the red

[1] This paper is the first of a series on the chemistry of the cotton plant.
[2] This work was done in cooperation with the Bureau of Entomology.
[3] Reference is made by number (italic) to "Literature cited," pp. 351–352.
[4] Perkins gives "rossrum," an evident typographic error for "roseum."

cotton flowers of *G. arboreum* L., from which, however, isoquercitrin and quercetin were isolated. Quercimeritrin, another glucosid, could be isolated only from one of the types mentioned—namely, the Egyptian cotton.

As a result of these investigations, the writers found that the petals of Upland cotton and the flowers with petals removed contained appreciable amounts of quercimeritrin. Very small amounts of isoquercitrin could also be found in the petals. So far, no gossypitrin or gossypetin could be isolated. The leaves were collected from plants grown at Brownsville, Texas, and the flowers from plants at Tallulah, Louisiana.

The results showing the character and distribution of color substances in the different types of the cotton plant are compiled in Table I, which shows clearly the chemical distinction among the different types of the cotton plant, as recently indicated by Perkin.

TABLE I.—*Distribution of glucosids and their products of hydrolysis in cotton*

Common name.	Scientific name.	Part examined.	Glucosids.		Product of hydrolysis.	Glucosid.	Product of hydrolysis.
			Quercimeritrin.	Isoquercitrin.	Quercetin.	Gossypitrin.	Gossypetin.
Egyptian cotton	*Gossypium barbadense.*	Flowers	Present.	Present.	Present.	Present.	Present.
Upland cotton	*G. hirsutum*	Leaves	do		do		
Do	do	Flowers with petals removed.	do				
Do	do	Petals	do	Present.			
Common yellow Indian cotton.	*G. herbaceum*	Flowers		do	Present.	Present.	Present.
Yellow Indian cotton.	*G. neglectum*	do		do	do	do	Do.
Red cotton	*G. arboreum*	do		do	do		
Pink cotton	*G. sanguineum*	do			do		
White Indian cotton.	*G. neglectum* var. *roseum.*	do					

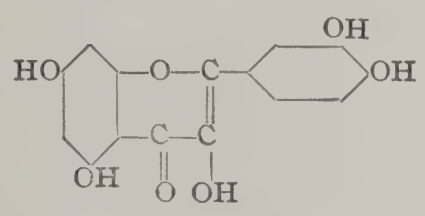
For the sake of comparison, the chief properties of the glucosids isolated from the cotton plant and their products of hydrolysis, compiled largely from the work of Perkin, are described below.

The glucosids quercimeritrin and isoquercitrin are isomeric compounds with the formula $C_{21}H_{20}O_{12}$. Isoquercitrin (*3*) has been found only in the cotton plant, while quercimeritrin (*1*) has also been found associated with prunitrin in the bark of a species of Prunus, closely related to *Prunus emarginata.*

QUERCIMERITRIN (*3*) crystallizes with three molecules of water from pyridin and water in small glistening plates of a bright-yellow color. They melt in a very pure state at 247° to 249° C., are almost insoluble in cold water or alcohol, but are rather easily soluble in the hot solvents. The acetyl derivative consists of needles that melt at 214° to 216° C. and are difficultly soluble in boiling alcohol.

ISOQUERCITRIN (*3*) crystallizes from pyridin and water in pale-yellow needles that melt at 217° to 219° C., are almost insoluble in cold water, sparingly soluble in boiling water. The acetyl derivative is much more soluble in alcohol than that of quercimeritrin. By hydrolysis both glucosids are split into quercetin and dextrose. Isoquercitrin is more easily hydrolyzed than is quercimeritrin.

QUERCETIN, a product of hydrolysis of quercimeritrin and isoquercitrin, is a flavone derivative to which the following formula has been assigned by various investigators (*10*):



Quercetin (*9*) has been found quite abundantly in various plants, either free or combined. Since some of these quercetin compounds are easily hydrolyzed, the finding of free quercetin may often be due to the occurrence of hydrolysis during the extraction. Most of the quercetin compounds are glucosids, the nature of the glucosid depending on the sugar groups with which quercetin is combined and also on the place of linkage. Among such glucosids not occurring in the cotton plant, quercitrin (*9*), rutin (*9*), and serotrin (*6*) have been isolated. The sugars obtained by the hydrolysis of these glucosids are dextrose and rhamnose.

Quercetin crystallizes from aqueous solutions with two molecules of water in fine needles of a bright-yellow color. It melts with partial decomposition at about 310° C.[1] It is only slightly soluble in boiling, and almost insoluble in cold water.

The solubility in boiling alcohol is 1 to 18, being more than 10 times the solubility in cold alcohol. The acetyl derivative crystallizes in colorless needles that melt at 194°.

GOSSYPITRIN[2] (*3*), another glucosid found in cotton, has the formula $C_{21}H_{20}O_{13}$. It crystallizes in small glistening needles of an orange-yrllow color and melts at 200-202°C. It is only slightly soluble in water and alcohol. The acetyl derivative crystallizes from acetic anhydrid and alcohol in colorless needles melting at 226° to 228° C. They are almost insoluble in alcohol.

[1] There is some confusion in the literature concerning the melting point of quercetin, some authors (*7 p. 524*) stating that it melts at 251° C., others (*8, p. 813*) at 310°. The writers found that their quercetin melted at 310°. The same was true of a commerical sample. Both of these samples of quercetin gave acetyl-quercetin melting at 194°.

[2] The presence of this glucosid in *Hibiscus sabdariffa* and *Thespasia lampas* has been reported by Schmidt, but the authors have been unable to locate the original references. (SCHMIDT, Ernst. AUSFÜHRLICHES LEHRBUCH DER PHARMAZEUTISCHEN CHEMIE. Aufl. 5, Bd. 2, p. 2040. Braunschweig, 1911.)

GOSSYPETIN.—By the hydrolysis of gossypitrin Perkin obtained gossypetin and a sugar identified as dextrose. Gossypetin has also been found as a glucosid in *Hibiscus sabdariffa* (*2*). It crystallizes in yellow needles melting at 311° to 313° C. They are slightly soluble in water, but are easily soluble in alcohol. Acetylgossypetin crystallizes from a mixture of alcohol and acetic acid in colorless needles melting at 228° to 230° C. These are readily soluble in acetic acid, but are somewhat sparingly soluble in alcohol.

EXPERIMENTAL WORK

ISOLATION OF QUERCIMERITRIN FROM THE PETALS

Quercimeritrin was isolated by Perkin's method (*3*), which was modified by omitting the use of lead acetate and was then employed in the following manner: An alcoholic extract from 1,214 gm. of air-dried, powdered petals was evaporated to a volume of about one and one-half liters. The deep purple-red solution was filtered from the dark material which had separated. This residue was warmed with water and filtered. On standing, an amorphous brick-red substance separated. This was filtered off by suction, boiled in absolute alcohol, and again filtered hot. On cooling, the yellow glucosid deposited in microcrystalline form, melting at 243° C. When mixed with known material obtained from petals by Perkin's original method, no lowering of the melting point was observed.

ISOLATION OF ISOQUERCITRIN FROM THE PETALS

In another experiment the alcoholic solution was evaporated to a small volume, and water was added. The mixture was then evaporated until most of the alcohol was gone. After extracting the aqueous solution with ether, neutral lead acetate was added. The heavy, dull-yellow precipitate produced by this reagent was filtered off. A thin paste was then made with water, and the lead removed by passing hydrogen sulphid through the mixture. After filtering, the filtrate was evaporated somewhat and allowed to stand in a vacuum desiccator for several days, when small greenish-yellow needles separated. On recrystallization from dilute methyl alcohol several times, the product finally melted at 247° C. The substance was undoubtedly quercimeritrin.

The filtrate from the lead acetate precipitation was treated with basic lead acetate, which produced another heavy dull-yellow precipitate. The lead was removed from this precipitate as in the previous case, and the solution was slowly evaporated over sulphuric acid in a vacuum desiccator. From this solution there was obtained by fractional crystallizations more quercimeritrin, and from the mother liquors a crystalline substance melting, after purification from pyridin and water, at 219° C. Hydrolysis of this compound yielded quercetin. It was consequently identified as isoquercitrin.

ISOLATION OF QUERCIMERITRIN FROM THE LEAVES

The leaves were treated according to Perkin's original method, as follows: Air-dried, pulverized cotton leaves (1,500 gms.) were heated with alcohol in an aluminum kettle for 4 hours on a steam bath. After cooling, the extract was pressed out and the dark-green liquid evaporated in a vacuum still to a small volume. Hot water was then added, and the distillation was continued until all the alcohol was removed. The hot mixture was then allowed to stand for about an hour, when an upper layer of black tar formed. The warm aqueous solution underneath was siphoned off and filtered through paper pulp. To remove chlorophyll and waxy matter, the cooled siphonate was twice extracted with ether, and the resulting clear, red, aqueous solution heated to expel any remaining ether. Lead-acetate solution was then added, and the thick orange-yellow precipitate that formed was filtered off by suction and washed with water. The lead was then removed from this precipitate by mixing it with hot water to make a thin paste, through which hydrogen sulphid was passed. The lead sulphid was filtered off by means of paper pulp. The clear, red filtrate was evaporated to a small volume and allowed to stand for several days, when a yellow amorphous substance slowly separated. Several fractions of this material were further obtained from the mother liquor. These fractions consisted of much quercetin and a little quercimeritrin. By a series of recrystallizations from water dilute alcohol, dilute acetic acid, and from pyridin, the quercimeritrin was obtained in a comparatively pure state. The quercimeritrin thus obtained melted at 247° C. When hydrolyzed with 5 per cent sulphuric acid, it gave quercetin. The latter compound was identified by the fact that it gave an acetyl derivative melting at 194° C. A mixture of quercimeritrin from the petals and from the leaves also melted at 247° C.

ISOLATION OF QUERCIMERITRIN FROM THE FLOWERS WITH PETALS REMOVED

These parts of the plant, treated in the same manner as the leaves, also gave quercimeritrin melting at 247° C.

II.—THE ETHEREAL OIL

While a volatile oil had previously been isolated in small amounts from the bark of the root of *Gossypium herbaceum* by Power and Browning (5), none had been reported from aboveground parts of this plant nor of other species of Gossypium.

The first intimation that a volatile oil might be present in plants of the Upland cotton was obtained by observing that a steam distillate of the leaves proved to be attractive to the boll weevil. The amount of oil found in the leaves or young plants was very small. In over 4,000 pounds of fresh plants only about an ounce of volatile oil was secured, amounting to 0.0015 per cent on an average.

The highest results from plants so far examined were yielded by squaring plants—namely, 0.0054 per cent in one and 0.0071 in another case. However, not sufficient data are on hand to allow any definite conclusions as to the relative yield from plants in different stages of growth.

The yield from the Sea Island cotton was about the same as that found in Upland cotton. The oil was of a more or less brown color, distilling over mainly between 200° and 300° C. at atmospheric pressure, the lower fractions having a yellow, the higher a blue color. The volatile oil obtained from cotton bark by Power and Browning had a pale-yellow color and distilled between 120° and 135° under the same conditions.

The oil isolated by the writers did not give any furfurol reaction nor deposit any crystals, and was also in these respects different from the oil isolated by Power and Browning, which gave a furfurol reaction and deposited crystals that crystallized from ethylacetate as needles melting at 112° to 114°, and consisted apparently of acetovanillon. Table II is of interest, since it shows the character and amount of plants used and the amount of oil yielded.

TABLE II.—*Results of distillations of cotton plants*

Date.	Weight of plants.	Oil yield. Weight.	Percentage of plant weight.	Description of plants.
	Pounds.	*Gm.*		
May 2 and 3	1,444	2.933	0.0004	Seedlings.
May 4	24	.586	.0054	Squaring plants from hotbed.
May 5	530	4.253	.0017	Seedlings.
May 8	86	.765	.0017	Do.
Do	87	.240	.0006	Do.
May 9	160	1.347	.0018	Do.
Do	124	1.194	.0021	Do.
May 21	25	.409	.0036	Do.
May 26	55	.380	.0015	Do.
May 31	431	7.536	.0038	Do.
June 1	4	.130	.0071	Squaring from hotbed.
June 4 and 5	528	3.760	.0016	Sea Island cotton.
June 6 and 11	624	5.400	.0019	Field cotton squaring.
Total of lots	4,122	28.933	.....	
Average of lots	.....	.....	.0015	

EXPERIMENTAL WORK [1]

The fresh plants, collected in the vicinity of Tallulah, La., were distilled as soon as possible with steam in a specially constructed apparatus. The distillate was shaken out with ether and the ether removed by placing the container in warm water, finally using water of about 50° to 60° C. All ether was considered removed when the oil showed little or no loss in weight after a few minutes' heating at this temperature.

[1] The kind assistance of Mr. L. A. Sallinger, of the Savannah Laboratory, and Dr. A. R. Albright, of the Food Investigation Laboratory, is hereby gratefully acknowledged.

In the experiment concerned with the fractional distillation, 7.84 gm. were heated on the steam bath for two hours. At the end of that time the oil had apparently lost weight to the extent of 0.04 gm., the loss consisting of ether. The heated sample was then fractionated at ordinary pressure, giving the following results.

Fraction.	Quantity of distillate.	Temperature of distillate.	Color.
		°C.	
1	4 to 6 drops	Up to 200	Light-yellow.
2	About 1 c. c	200–250	Greenish yellow.
3	About 1 to 2 c. c	250–270	Light blue-green.
4	2 to 3 c. c	270–285	Dark blue-green.
5	Less than 1 c. c	285–300	Dark blue.
6	Few drops	300–325	Darker blue
Residue	About 1 c. c		Black.

The odor of the residue only seemed empyreumatic.

SUMMARY

(1) Quercimeritrin and isoquercitrin, formerly isolated from other types of the cotton plant, have now also been found in Upland cotton (*Gossypium hirsutum*).

(2) The leaves and flowers, with petals removed, contained quercimeritrin, while the petals contained both quercimeritrin and isoquercitrin.

(3) No traces have been found of gossypitrin and gossypetin, which have been isolated from other types of cotton.

(4) An ethereal oil has been isolated from *G. hirsutum* which is different from that found in the bark of the root of *G. herbaceum*. It distills mainly between 200° and 300° C., and leaves a black empyreumatic residue. The lower fractions of the distillate have a yellow to greenish-yellow color, the higher fractions light blue-green to dark blue. This oil proved to be attractive to the boll weevil.

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STABILITY OF OLIVE OIL[1]

By E. B. Holland, *Associate Chemist*, and J. C. Reed and J. P. Buckley, Jr., *Assistant Chemists*,[2] *Massachusetts Agricultural Experiment Station*

INTRODUCTION

Some years ago one of the writers reported the results of an experiment to determine the effect (*4*)[3] of air, light, and moisture at room temperature on butter fat. The test was planned to show the action of the three agents, singly and in combination, and was continued for a year and a half. The experiment furnished considerable information relative to the changes that take place in such materials, but proved faulty in that a fat, solid and opaque at ordinary temperature, was a poor medium for measuring such changes, which evidently were not uniform throughout the mass, but greatest at the surface; furthermore, the conditions surrounding the fat were not under satisfactory control.

Conceding the limitations of the previous experiment, but recognizing the economic value as well as scientific interest of such investigations, the writers deemed it advisable to conduct another series of tests, under more definite conditions. For this purpose all oils procurable in quantity at a reasonable price were carefully considered. Olive oil was finally selected for the reason that it is a well-known edible product of fair keeping properties and of the composition desired.

THE OIL EMPLOYED

A few letters of inquiry to Federal and Experiment Station officials elicited the information that pure olive oil, both foreign and domestic, was readily obtainable. As an American oil could be procured directly from the pressers in a comparatively short time, with details of production and treatment, an order was placed, specifying an absolutely pure product that had not been bleached, sterilized, or refined in any way except as to filtration and having a low content of free fatty acids.

A 5-gallon can of California olive oil was received on February 25, 1910. The manufacturer stated that the oil was cold-pressed from an average run of hand-picked, washed, and ground ripe California-grown olives of the season of 1908. After extraction, the oil was pumped into settling tanks and from there to storage tanks, whence it was filtered four to six times through French filter paper in a special press and was not put on the market until it was at least a year old.

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[1] From the Department of Chemistry, Massachusetts Agricultural Experiment Station. Printed with the permission of the Director of the Station.

[2] Mr. Reed was associated with the senior writer in the earlier stages of the work and Mr. Buckley in the later.

[3] Reference is made by number (italic) to "Literature cited," p. 366.

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ORGANOLEPTIC TESTS

The appearance of the oil as determined by the unaided eye varied with the depth of stratum and character of the light from transparent olive-green to opaque, almost black. All efforts at color differentiation without an instrument proved unsatisfactory, but were continued throughout the experiment, as a tintometer was not available for the first four years. In amounts of 6 ounces, the basis employed, the oil will be designated a dark olive-green. The green greatly exceeded that in most olive oils offered in local markets, probably due, as the manufacturer claims, to differences in soil and climatic conditions, together with possibly small variations in manufacturing methods. The oil had an excellent body and a pronounced olive odor.

PHYSICAL TESTS

Specific gravity $\frac{20^\circ}{20^\circ}$ C.		0.91308
Specific gravity $\frac{20^\circ}{4^\circ}$ C. (calculated)		0.91152
Specific gravity 25° C. (U. S. P. standard)	0.910–	0.915
Refractive index $n \frac{20^\circ \text{ C.}}{D}$ (Abbe)		1.4687
Viscosity 70° F. (Redwood)		12.0
Valenta test (B. and A. 99.5 per cent acid)		87.5° C.
Elaidin test		Green, semisolid

CHEMICAL TESTS

Saponification number		190.636
Saponification number (U. S. P. standard)	190–195	
Acid number (a)		1.990
Ether number (e)		188.646
Total fatty acids (1.00–0.00022594e)		95.74 per cent
Neutralization number (n)		199.12
Mean molecular weight		281.78
Free fatty acids as oleic acid and as $\frac{a}{n}$		1.00 per cent
Glycerol (0.00054703e)		10.32 per cent
Reichert-Meissl number		None
Polenske number		0.13
Insoluble acids		95.40 per cent
Iodin number (Wijs)		83.45
Iodin number (U. S. P. standard)	79– 90	
Acetyl number		5.69

COLOR TESTS

Baudouin test for sesame oil	Nil
Bechi silver nitrate test for cottonseed oil	Nil
Halphen test for cottonseed oil	Nil
Nitric-acid test for seed oils	Brown, slight coagulation

All organoleptic, physical, chemical, and color tests indicated a pure olive oil, with the exception of the nitric-acid test which may be disregarded, as it is no longer designated by the Pharmacopœia.

PLAN OF THE EXPERIMENT

The object of the investigation primarily was to ascertain the nature and extent of the action of the several agents upon the oil as determined by changes in physical characteristics and chemical composition; and secondarily to deduce, if possible, from the results obtained a practical method for handling commercial oils. The experiment was planned to demonstrate the effect of air, light, and moisture, singly and in combination, which, together with control (the basis for comparison) and enzym-free samples, required nine series of tests, as follows:

Series.	Condition of the experiment.	Series.	Condition of the experiment.
A	Control.	F	Air-light.
B	Enzym-free.	G	Air-moisture.
C	Air.	H	Light-moisture.
D	Light.	I	Air-light-moisture.
E	Moisture.		

As the change in the oil in most cases would be comparatively slow, six years were believed necessary to obtain the maximum effect desired. The previous experiment having demonstrated that analysis oftener than once a year did not compensate for the extra labor involved, only 1 sample was alloted for each year, or 6 for each series, making a total of 54 samples. Six ounces of oil were taken for each sample which was insufficient for some physical tests, but ample for most chemical. Round flint-glass bottles of 6-ounce capacity with glass stoppers were used as containers after being carefully cleaned and dried.

Five c. c. of distilled water were pipetted into each bottle of series E, G, H, and I, after which all the bottles, with the exception of series B, were filled with the oil as received, after it was thoroughly mixed to insure uniformity. Another portion of the oil, used for series B, was heated to 70° C. on two successive days for approximately 60 minutes on the first day and 30 on the second, to destroy enzyms, if any were present. All bottles were filled to the shoulder. Where air was not a factor, the bottles were closed with glass stoppers and carefully sealed with wax. Such treatment failed as a control measure, as a small amount of air remained in the bottles; but this appeared unavoidable under the circumstances. Each series of tests was inclosed in an 8-inch Fruehling and Schultz desiccator after the porcelain plate had been removed. The bottles in an upright position were arranged in a circle and well spaced.

To exclude the action of moisture (series A, B, C, D, and F) sulphuric acid, previously heated in most cases to 212° C., or higher, was poured into the desiccators to absorb any water that might gain access. To secure a saturated atmosphere (series E, G, H, and I), distilled water was poured into the desiccators, in addition to the water in the bottles.

To exclude air (series A, B, D, E, and H), the desiccators were rarefied by means of a vacuum pump, and a small U-shaped manometer was suspended from the hook of the stopcock to indicate the rarefaction and its

permanence. The joints of these desiccators were covered with wax; but even under the best conditions leakage could not be prevented entirely, and it was found necessary to pump out the desiccators several times a year. To obtain the effect of air (series C, F, G, and I) the glass stopcocks of the desiccators were replaced by perforated rubber stoppers and straight glass tubes which passed through the stoppers and dipped into the sulphuric acid (series C and F) or into the water (series G and I).

To exclude light (series A, B, C, E, and G), the desiccators were placed in a large oblong wooden box, lined with building paper, with an overhanging cover similarly lined. The cover was held by corner posts 0.5 inch above the top of the box, projected 0.5 inch beyond the sides of the box, and overlapped 3.5 inches. In addition a strip 1.4 inches wide was nailed to the outside of the box 0.5 inch below the edge of the cover.

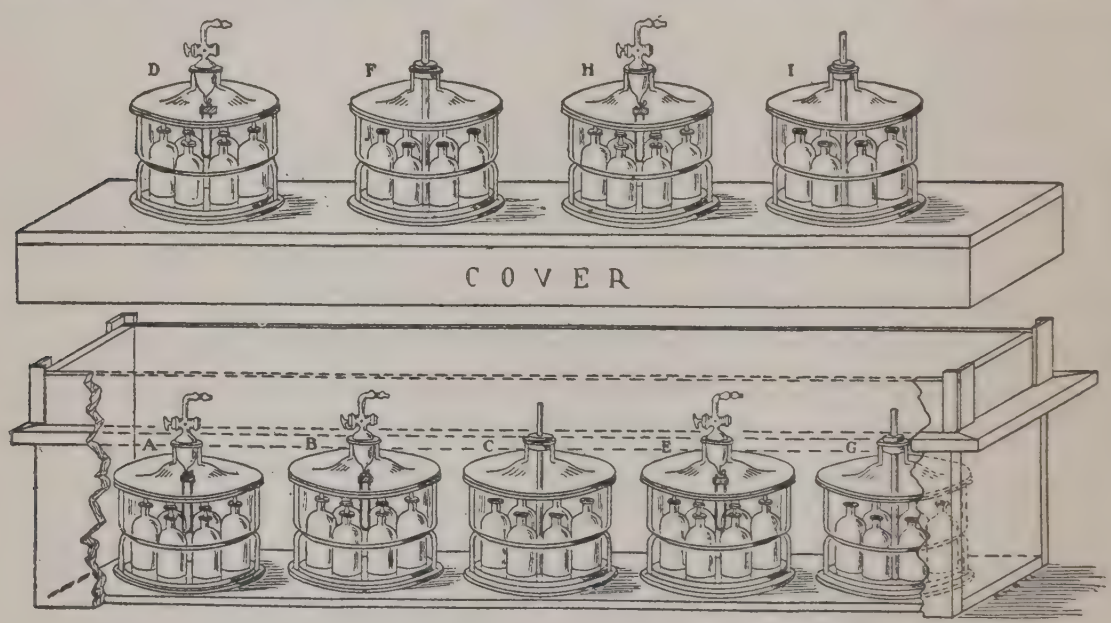

FIG. 1—Apparatus used in the experiments to determine the stability of olive oil.

This provided a continuous air passage 0.5 inch wide under all sides of the cover and yet absolutely prevented the entrance of light, even by reflection. To obtain the effect of light (series D, F, H, and I) the desiccators were placed on the cover of the box and exposed to light from a north window. All the samples were kept in the northeast room of the Experiment Station dairy building, into which the direct rays of the sun did not enter at any season of the year. The temperature was not constant, but relative in all cases. Considerable time was consumed in obtaining the necessary supplies and in preparing the samples, so that the experiment did not actually begin until April 2, 1910.

EFFECT OF AIR, LIGHT, AND MOISTURE

ORGANOLEPTIC CHANGES

Changes of an organoleptic character are difficult to measure and even more difficult to express, particularly where the differences are slight. The results are relative, however, if not strictly accurate, and are recorded in Table I.

TABLE I.—*Organoleptic changes in the olive oil*

Series.	Conditions of the experiment.	As received (1910).	After 1 year (1911).	After 2 years (1912).	After 3 years (1913).	After 4 years (1914).	After 5 years (1915).	After 6 years (1916).
A	Control	Clear; dark olive green; marked olive odor.	Clear; dark olive green; olive odor.	Clear; dark olive green; color and olive odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.	Clear; olive green; color and odor gradually diminish.
B	Enzym-free	do	do	do	do	do	do	Do.
C	Air	do	do	do	Less color than A; less odor than A.	Less green than A; slightly rancid odor (?).	Less green than A	Yellowish; rancid odor.
D	Light	do	Less green than A.	Less green than A	Greenish yellow	Greenish yellow	Slightly greenish yellow.	Slightly greenish yellow; slightly rancid odor.
E	Moisture	do	Turbid with small amount of precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with more precipitate than G, after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.	Turbid with some precipitate; after filtering, color like A.
F	Air-light	do	Less green than D.	Light yellow, color largely destroyed; rancid odor.	Yellowish, nearly colorless; rancid odor.	Yellowish, nearly colorless; rancid odor.	Nearly colorless; rancid odor.	Nearly colorless; rancid odor.
G	Air-moisture	do	Clear with slight amount of precipitate; after filtering, color like A.	Clear with some precipitate, like E; after filtering, color like A.	Clear with least precipitate; after filtering, color like A; water acid to litmus; odor like C.	Clear with some precipitate, like E; after filtering, less green than C.	Slightly turbid with least precipitate; color less than D; rancid odor.	Slightly turbid with less precipitate than E; after filtering, slight color; rancid odor.
H	Light-moisture	do	Turbid with more precipitate than E; after filtering, color between D and F.	Turbid with more precipitate than G; after filtering, color lighter than D.	Turbid with more precipitate than E; after filtering, color like D; slightly rancid odor (?).	Turbid with considerable precipitate; after filtering, color between D and F.	Turbid with more precipitate than E; after filtering, more yellow than D.	Turbid with more precipitate than E; after filtering, color like D.
I	Air-light-moisture	do	Turbid with more precipitate than H; after filtering, color between H and F.	Turbid with most precipitate; after filtering, color like F; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless like F; water acid to litmus; rancid odor.	Turbid with most precipitate; after filtering, less color than F; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless; rancid odor.	Turbid with most precipitate; after filtering, nearly colorless; rancid odor.

The color and characteristic odor of the oil seemed to diminish gradually in the control samples. The heated samples, series B, duplicated the control samples, so far as could be observed.

Air was a negligible factor for two years; then it effected a slow but marked destruction of color fully equal to light at the close of the experiment, and caused a rancid odor on the sixth year.

Light was active in destroying color and caused a slightly rancid odor on the sixth year, probably due to a small amount of inclosed air.

Moisture caused the formation of a precipitate which rendered the oil turbid, but which effected no apparent change in color after the removal of the precipitate.

Air-light was most active and effective in destroying color, equal to air-light-moisture, and produced a rancid odor on the second year.

Air-moisture caused the formation of a slight amount of precipitate but without appreciable turbidity until the fifth year, at which time a rancid odor was produced. Air-moisture was inactive as regards color for three years, but eventually exceeded the effect of air and equaled that of light.

Light-moisture affected the color about the same as light, and caused the formation of a considerable amount of precipitate which rendered the oil turbid.

Air-light-moisture affected the color the same as air-light, produced a rancid odor the second year, and caused the formation of probably the largest amount of precipitate, which rendered the oil turbid.

The chromogenic bodies of the oil were not appreciably affected by moisture, were destroyed slowly but effectively by air, slowly but rather more effectively by air-moisture, more actively by light and light-moisture, and most actively and effectively by air-light and by air-light-moisture. Air was slowly active, light probably assisted by a small amount of inclosed air more active, and air-light the most active in destroying color. Moisture was a negligible factor except possibly in the case of air-moisture.

A rancid odor was produced on the sixth year by air and by light, on the fifth year by air-moisture, and on the second year by air-light and by air-light-moisture. Neither air nor light alone was particularly active in producing rancidity, but jointly were decidedly effective. In this connection moisture did not appear to be a factor of any consequence.

In every instance the presence of moisture caused the formation of a precipitate in a relatively slight amount by air-moisture, in small amount by moisture, in a greater amount by light-moisture, and in apparently the largest amount by air-light-moisture. Light seemingly was a factor. The so-called precipitate was first observed as dirty-white or brownish-white spots on the sides of the bottle below the surface of the oil and might be said to resemble mold. As the amount increased, the bulk of it collected near the surface of the water layer or in the water. In no case was sufficient purified material obtained to make a chemical examination.

PHYSICAL TESTS

The refractive index of the different series was determined for a number of years by means of an Abbe refractometer. The readings did not indicate any appreciable change in the oil except with air-light and with air-light-moisture, where gains of approximately 0.001 were noted. The results for the year 1912 corrected are given in Table II. Those for 1913 and 1914 gave like differences and are not reported.

TABLE II.—*Refractive index for the olive oil, 1912*

Series.	Conditions of the experiment.	$n \frac{20° C.}{D}$
A	Control	1.4687
B	Enzym-free	1.4687
C	Air	1.4690
D	Light	1.4690
E	Moisture	1.4690
F	Air-light	1.4700
G	Air-moisture	1.4689
H	Light-moisture	1.4689
I	Air-light-moisture	1.4702

A Lovibond tintometer was employed for determining the color of the oil in 1915 and 1916. The supply of standard glasses in 1915 was inadequate for satisfactory readings, particularly for the darker oils, and the results are merely indicative.

TABLE III.—*Color of the olive oil*

Series.	Conditions of the experiment.	Stratum.	Matching standards.			Color developed.	
			Red.	Yellow.	Blue.	Orange.	Yellow.
	September, 1915.						
		Inches.					
A	Control	0.50	1.0	6.8	.....	1.0	5.8
B	Enzym-free	.50	1.0	11.0	.....	1.0	10.0
C	Air	1.00	.5	1.4	.....	.5	.9
D	Light	.50	1.0	3.5	.....	1.0	2.5
E	Moisture	.50	1.0	7.3	.....	1.0	6.3
F	Air-light	1.00	.3	.8	.....	.3	.5
G	Air-moisture	1.00	.5	1.3	.....	.5	.8
H	Light-moisture	.50	1.0	3.5	.....	1.0	2.5
I	Air-light-moisture	1.00	.1	.7	.....	.1	.6
	March, 1916.						
A	Control	.25	1.0	12.8	.....	1.0	11.8
B	Enzym-free	.25	1.0	12.8	.....	1.0	11.8
C	Air	1.00	.7	1.8	.....	.7	1.1
D	Light	.50	.8	3.5	.....	.8	2.7
E	Moisture	.25	1.0	12.8	.....	1.0	11.8
F	Air-light	2.00	.5	1.5	.....	.5	1.0
G	Air-moisture	1.00	.2	1.8	.....	.2	1.6
H	Light-moisture	.50	1.2	4.6	.....	1.2	3.4
I	Air-light-moisture	2.00	.5	1.5	.....	.5	1.0

According to the tintometer readings, for the last two years (1915–16), the control, enzym-free, and moisture samples retained the most color; light and light-moisture next; air and air-moisture less; and air-light and air-light-moisture the least color. The organoleptic tests for the entire period rated light, and light-moisture more active than air and air-moisture, but equally effective at the close of the experiment, or nearly so. Differences between organoleptic and tintometer readings are due, partly at least, to the fact that the unaided eye is less sensitive to the yellow than to the darker colors and is unable to differentiate accurately between faint colors, but more particularly to failure in properly coordinating activeness or speed of destruction and effectiveness or completeness of destruction.

The viscosity of a "fractional" quantity of several of the samples was determined in 1912 by means of a Redwood viscosimeter.

TABLE IV.—*Viscosity of the olive oil, 1912*

Series.	Conditions of the experiment.	Viscosity.
A	Control	15.8
F	Air-light	20.5
I	Air-light-moisture	21.7

Air-light and air-light-moisture evidently increased the viscosity to a slight extent.

CHEMICAL TESTS

The decomposition of the olive oil as affected by air, light, and moisture, singly and in combination, was measured in terms of acid, saponification, and iodin numbers. At the outset the oil seemed to possess a certain resistance to hydrolysis, oxidation, etc., but after it began to break down to any extent, the changes were more rapid. The hydrolytic effect of air, light, and moisture on the glycerids of the oil was measured in terms of acid number, which indicates the amount of free fatty acids produced (Table V).

TABLE V.—*Acid number of the olive oil*

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	1.99	2.18	+0.19	2.29	+0.30	2.47	+0.48	2.66	+0.67	2.97	+ .98	3.00	+1.01
B	Enzym-free	.....	2.18	+0.19	2.20	+0.21	2.28	+0.29	2.39	+0.40	2.68	+ .69	2.64	+ .65
C	Air	.....	2.07	+0.08	2.01	+0.02	2.03	+0.04	2.25	+0.26	2.60	+ .61	2.96	+ .97
D	Light	.....	2.26	+0.27	2.38	+0.39	2.54	+0.55	2.86	+0.87	3.06	+1.07	2.39	+ .40
E	Moisture	.....	2.33	+0.34	2.55	+0.56	2.93	+0.94	3.48	+1.49	4.15	+2.16	4.59	+2.60
F	Air-light	.....	2.10	+0.11	2.28	+0.29	2.78	+0.79	3.58	+1.59	4.94	+2.95	6.83	+4.84
G	Air-moisture	.....	2.35	+0.36	2.60	+0.01	2.97	+0.98	3.69	+1.70	4.60	+2.61	5.07	+3.08
H	Light-moisture	.....	2.46	+0.47	2.81	+0.82	3.29	+1.30	4.01	+2.02	4.86	+2.87	5.59	+3.60
I	Air-light-moisture	.....	2.42	+0.43	3.09	+1.10	4.08	+2.09	5.88	+3.89	8.09	+6.10	12.45	+10.46

The control samples (the basis for comparison) hydrolyzed a little more than the enzym-free, although the differences were slight.

Neither air nor light showed any appreciable action.

Moisture was moderately active and gradually effected a noticeable amount of hydrolysis.

Air-moisture and light-moisture were rather more effective than moisture, although the influence of the air or of the light must have been secondary.

Air-light was inactive for two years; then it began to affect hydrolysis and eventually exceeded air-moisture and light-moisture, probably due to the impossibility of entirely excluding moisture under the conditions of operation.

Air-light-moisture was the first to effect an appreciable amount of hydrolysis and greatly exceeded all others at the close.

Moisture effected considerable hydrolysis; air-moisture and light-moisture caused an additional amount; air-light, probably assisted by some moisture, still more, and air-light-moisture was the most active and effective. Moisture was the essential factor although air and light together greatly accelerated it.

The decomposition of unsaturated acids of olive oil as effected by air, light, and moisture may be measured in a degree by the increase in the saponification number which indicates the amount of fatty acid of high molecular weight converted into acids of lower molecular weight (Table VI).

TABLE VI.—*Saponification number of the olive oil*

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	190.64	189.86	−0.78	189.25	−1.39	190.53	−0.11	190.62	−0.02	190.57	− 0.07	190.07	− 0.57
B	Enzym-free	.....	190.18	−0.46	189.98	−0.66	190.62	−0.02	190.79	+0.15	190.80	+ 0.16	190.60	− 0.04
C	Air	.....	190.09	−0.55	190.26	−0.38	190.73	+0.09	192.24	+1.60	194.01	+ 3.37	195.36	+ 4.72
D	Light	.....	189.89	−0.75	189.58	−1.06	189.94	−0.70	189.88	−0.76	190.37	− 0.27	190.45	− 0.19
E	Moisture	.....	189.87	−0.77	189.18	−1.46	190.12	−0.52	190.03	−0.61	190.68	+ 0.04	189.80	− 0.84
F	Air-light	.....	190.58	−0.06	191.79	+1.15	194.55	+3.91	195.10	+4.46	201.74	+11.10	203.68	+13.04
G	Air-moisture	.....	189.92	−0.72	190.19	−0.45	190.93	+0.29	191.84	+1.20	194.40	+ 3.76	194.77	+ 4.13
H	Light-moisture	.....	189.81	−0.83	189.89	−0.75	189.91	−0.73	189.64	−1.00	189.41	− 1.23	189.82	− 0.82
I	Air-light moisture	.....	190.44	−0.20	192.28	+1.64	194.07	+3.43	196.16	+5.52	200.16	+ 9.52	203.85	+13.21

The control, enzym-free, light, moisture, and light-moisture samples were not affected in total alkali-consuming power. Air and air-moisture caused a like increase in saponification number.

Air-light and air-light-moisture effected a greater increase and of like amount. Air was undoubtedly the principal factor, although greatly intensified by light.

Fittig (*1-3*) has shown that unsaturated acids with the double bond in $\beta\gamma$, or 9–10 position, as in oleic acid, may undergo intramolecular changes on boiling with caustic alkali, forming an α-β, or 2-3 acid, which on fusion with alkali, according to Varrentrapp (*9, p. 209–215*) and Molinari (*6, p. 293*), splits into acetic and another saturated acid.

$$\underset{\text{9-10 oleic acid}}{CH_3.(CH_2)_7.CH : CH.(CH_2)_7.COOH} = \underset{\alpha\text{-}\beta \text{ or 2-3 oleic acid.}}{CH_3.(CH_2)_{14}.CH : CH.COOH}$$

$$CH_3.(CH_2)_{14}.CH : CH.COOH + 2\ KOH = \underset{\text{palmitate}}{CH_3.(CH_2)_{14}.COOK} + \underset{\text{acetate.}}{CH_3COOK} + H_2$$

Schrauth (*7*) confirmed the reaction and claimed further that in general for each double bond two carbon atoms are split off in the form of acetic acid. Different authorities (*5, p. 353–354*) have shown that the unsaturated (liquid) acids of olive oil consist principally of oleic acid with a smaller amount of linolic acid.

After considering the high content of unsaturated acids and their possible decomposition as described, one might suggest as a tentative hypothesis that the increase in saponification number was due to the formation of a α-β, or 2-3 acid, from the action of air or more effectively from air-light, which on boiling with alcoholic potash broke down into acetic and palmitic acids. Either oleic or linolic acid might be affected, although the latter is unquestionably less stable and therefore more likely to undergo molecular change. Both oleic and linolic acids, on breaking down into acetic and palmitic acids, double their alkali-consuming power; therefore, the increase in saponification number would be directly proportional to the amount of acid affected. The statement made by Schrauth that one molecule of acetic acid splits off for each double bond, appeared untenable for linolic acid in this connection. The neutralization number of oleic acid is 198.709 and of linolic acid 200.138. Assuming that α-β, or 2-3 oleic acid and linolic acid, after molecular rearrangement have no appreciable iodin numbers, as determined by Wijs solution, the writers obtained the following results for the sixth year of the experiment (Table VII):

TABLE VII.—*Character of the decomposition of the olive oil (1916)*

Series.	Conditions of the experiment.	Increase in saponification number.	Equivalent to oleic acid.	Equivalent loss in iodin number.	Equivalent to linolic acid.	Equivalent loss in iodin number.	Actual loss in iodin number.[1]
			Per cent.		*Per cent.*		
C	Air	4.72	2.375	2.135	2.358	4.270	3.85
F	Air-light	13.04	6.562	5.899	6.516	11.800	11.88
G	Air-moisture	4.13	2.078	1.868	2.064	3.738	3.99
I	Air-light-moisture	13.21	6.648	5.976	6.600	11.952	11.68

[1] See Table VIII.

The close agreement of the calculated and the actual loss of iodin numbers on the basis of linolic acid would indicate that probably only linolic acid had been affected during the term of the experiment. The matter will be considered further under iodin number (Table VIII).

The decomposing action of air, light, and moisture on the unsaturated acids of olive oil may also be measured by the loss in iodin number (Table VIII.

TABLE VIII.—*Iodin number of the olive oil*

Series.	Conditions of the experiment.	Test in 1910 (as received).	Test in 1911.	Change in 1 year.	Test in 1912.	Change in 2 years.	Test in 1913.	Change in 3 years.	Test in 1914.	Change in 4 years.	Test in 1915.	Change in 5 years.	Test in 1916.	Change in 6 years.
A	Control	83.45	83.97	+0.52	83.05	−0.40	82.99	−0.46	83.85	+0.40	83.86	+0.41	83.88	+ 0.43
B	Enzym-free	.....	83.84	+0.39	82.87	−0.58	83.09	−0.36	83.57	+0.12	83.97	+0.52	83.73	+ 0.28
C	Air	.....	83.79	+0.34	82.52	−0.93	82.58	−0.87	81.97	−1.48	81.17	−2.28	79.60	− 3.85
D	Light	.....	83.97	+0.52	82.84	−0.61	83.01	−0.44	83.88	+0.43	83.99	+0.54	83.67	+ 0.22
E	Moisture	.....	84.00	+0.55	83.17	−0.28	83.32	−0.13	83.80	+0.35	83.81	+0.36	83.79	+ 0.34
F	Air-light	.....	83.23	−0.22	80.80	−2.65	79.49	−3.96	77.27	−6.28	75.31	−8.14	71.57	−11.88
G	Air-moisture	.....	83.91	+0.46	82.79	−0.66	82.66	−0.79	82.43	−1.02	81.17	−2.28	79.46	− 3.99
H	Light-moisture	.....	83.97	+0.52	83.15	−0.30	82.95	−0.50	84.17	+0.72	83.99	+0.54	84.00	+ 0.55
I	Air-light-moisture	.....	83.29	−0.16	80.84	−2.61	79.80	−3.65	77.29	−6.16	75.20	−8.25	71.77	−11.68

The control, enzym-free, light, moisture, and light-moisture samples were not affected. Air and air-moisture caused considerable loss and of substantially the same amount. Air-light and air-light-moisture were equally effective and caused much greater loss than air or air-moisture; presumably moisture was a negligible factor in both instances. Air was the principal factor, although greatly intensified by light. The loss in iodin number was proportional to the gain in saponification number evidently two different measurements of the same decomposition, as shown by Table IX. The iodin number of linolic acid is 181.091.

TABLE IX.—*Character of the decomposition of the olive oil (1916)*

Series.	Conditions of the experiment.	Loss in iodin number.	Equivalent to linolic acid.	Equivalent gain in saponification number.	Actual gain in saponification number.[1]
			Per cent.		
C	Air	3.85	2.126	4.254	4.72
F	Air-light	11.88	6.560	13.129	13.04
G	Air-moisture	3.99	2.203	4.409	4.13
I	Air-light-moisture	11.68	6.450	12.909	13.21

[1] See Table VI.

The formation of aldehyde as a result of oxidation was manifested by the color imparted to the alcoholic potash in the determination of saponification number (Table X). The fuchsin-aldehyde reagent (7, *p. 15*) was employed as a confirmatory test, but proved rather too sensitive for the purpose.

TABLE X.—*Production of aldehyde in the olive oil*

Series.	Conditions of the experiment.	As received, 1910.	1911	1912	1913	1914	1915	1916
C	Air	.....	.....	.....	Trace	Present	Present	Present.
F	Air-light	.....	.....	Trace	Present	Considerable.	Considerable.	Considerable.
G	Air-moisture	.....	.....	.....	Trace	Present	Present, like C.	Present, less than C.
I	Air-light-moisture.	.....	.....	Trace; more than F.	Present	Considerable.	Considerable; less than F.	Considerable; more than F.

The relative amount of aldehyde present in the samples was estimated by the depth of color produced. Air and air-moisture produced a small amount of aldehyde, which was first noticed on the third year. Air-light and air-light-moisture produced a larger amount, first noticed on the second year. Evidently air was the essential factor accelerated by light.

The original olive oil was carefully examined for enzyms by Dr. G. H. Chapman, of this Station, and although their presence was not detected, it is impossible to say whether any of the changes noted were induced or accelerated by their action.

DISCUSSION OF RESULTS

Air effected no appreciable change in the color of olive oil for two years; then it caused a slow but marked destruction fully equal to light at the close. The tintometer for the last two years (1915–1916) showed air more destructive than light and light-moisture. Air alone was not active in producing rancidity, which was not noticeable until the sixth year. Air had no hydrolytic action but was the active factor in the decomposition of unsaturated acids and in the production of aldehyde.

Light was more active in destroying color than air or air-moisture, but according to the tintometer for the last two years it did not effect as complete destruction as air and air-moisture. Light alone was not active in producing rancidity, which was not noticeable until the sixth year, probably owing to a small amount of inclosed air. Light alone had no hydrolytic action on the glycerids or decomposing action on the unsaturated acids.

Moisture had no effect on the chromogenic bodies and did not appear a factor of any consequence in producing rancidity. Moisture caused the formation of a precipitate and a turbid oil. Moisture was the essential factor in hydrolysis and although only moderately active, gradually effected a considerable amount.

Air-light, like air-light-moisture, was the most active and effective in destroying color and in producing rancidity, which was noticeable on the second year. Air-light increased the refractive index and viscosity of the oil. Air-light had no hydrolytic action for two years; then it

gradually exceeded air-moisture and light-moisture, probably owing to the difficulty of entirely excluding moisture and at the same time permitting the entrance of air. Air-light effected more decomposition of unsaturated acids and production of aldehyde than air. Light evidently accelerated the action of air in this connection.

Air-moisture had no action on color for three years, but eventually exceeded air and equaled that of light in effectiveness. According to the tintometer, for the last two years air-moisture has exceeded light and light-moisture and has equaled that of air in destroying color. Air-moisture effected rancidity the fifth year, exceeding air and light. Air-moisture caused the formation of a slight amount of precipitate but no appreciable turbidity until the fifth year. Air-moisture effected more hydrolysis but the same amount of decomposition of unsaturated acids and formation of aldehyde as air.

Light-moisture was as effective in destroying color as light and more active than air or air-moisture. According to the tintometer, for the last two years light and light-moisture did not effect as complete destruction of color as air and air-moisture. Light-moisture caused the formation of more precipitate than moisture and a turbid oil. Light seemingly was a factor. Light-moisture effected more hydrolysis than moisture and as much as air-moisture.

Air-light-moisture, like air-light, was the most active and effective in destroying color and in producing rancidity, which was noticeable in the second year. Air-light-moisture caused the formation of apparently the most precipitate and a turbid oil. Air-light-moisture increased the refractive index and viscosity of the oil substantially the same as air-light. Air-light-moisture was the first to effect hydrolysis and exceeded all others in amount. Air-light-moisture effected the same decomposition of unsaturated acids and production of aldehyde as air-light, which greatly exceeded that of air or of air-moisture.

PRACTICAL DEDUCTIONS

From an economic standpoint air caused a slow destruction of color in olive oil, the production of rancidity, and the decomposition of unsaturated acids.

Light caused an active destruction of color and a slow production of rancidity.

Air-light caused the most active and effective destruction of color, active destruction of unsaturated acids, a rapid production of rancidity, and a slow but marked production of free fatty acids.

Moisture caused the production of a precipitate, a turbid oil, and free fatty acids.

Air-moisture practically duplicated the effect of air plus that of moisture, and light-moisture that of light plus that of moisture.

Air-light-moisture exceeded the effect of air-light plus that of moisture in the amount of free fatty acids produced; otherwise it was essentially the same.

In order to preserve olive oils in their natural state, air, light, and moisture should be excluded as completely as possible, particularly the combined action of air and light, which has proved exceedingly destructive.

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SOME BACTERIAL DISEASES OF LETTUCE

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INTRODUCTION

This paper is an attempt to classify some common bacterial softrots of lettuce (*Lactuca sativa*). The rots of lettuce, sometimes very destructive, have needed a critical study for a long time, but they have been rather neglected by plant pathologists, owing partly perhaps to their sporadic character but also partly to the fact that they are difficult to work with, as the soft, slimy character of the rotted tissues is somewhat repellent and also is prompt to invite confusing secondary invasions. Hitherto various softrots have been ascribed to bacteria, but mostly on insufficient evidence, and generally without a proper description of the supposed parasite. This paper deals with four outbreaks of lettuce rot in the United States—viz, (1) The Louisiana disease of 1915—already reported in a preliminary way by the writer (*8*)[1]; (2) the Beaufort (South Carolina) disease of 1916; (3) the Portsmouth (Virginia) disease of 1916; and (4) the Kansas disease of 1916. It also discriminates two new lettuce parasites (both Schizomycetes) and describes their morphological and physiological characters.

EARLIER LITERATURE

A short account of the literature on bacterial diseases of lettuce has been given in the paper (*8*) describing the Louisiana lettuce disease; therefore only an account of a disease of lettuce occurring in the Rio Grande Valley need be referred to.

Carpenter in 1916 gave a brief account (*9*) of an investigation of a lettuce disease occurring in the lower Rio Grande Valley. The general symptoms are those of a gradually dying plant. He describes the gross symptoms as follows: (1) A reddening of the older leaves and blanching of the younger central leaves; (2) a restricted development of newly forming leaves, accompanied by small dark-colored blister spots along the border; (3) the development of numerous lateral adventitious shoots; and (4) dry and dead small roots. Carpenter did not find any parasitic insects or fungi constantly associated with the disease. The symptoms indicated a root trouble, and he believed that the presence of alkali in the soil offered a partial explanation of the disease.

[1] Reference is made by number (italic) to "Literature cited," p. 388.

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THE SOUTH CAROLINA LETTUCE DISEASE

The South Carolina outbreak of lettuce-rot occurred in Beaufort County, the second largest lettuce-growing district on the eastern coast of the United States, with a reputation of growing the finest quality of Big Boston head lettuce on the entire eastern coast. The South Carolina disease may be either a stem or a leaf infection (Pl. 29, A, B). In an early stage the plants are a lighter green color than the healthy ones; later the head may show rot through the center or only on the top. A general wilting of the head may occur with or without visible spots or rot. In some cases rotting is rapid; in others the heart remains sound, while the outer encircling leaves are in a bad state of decay. The diseased plants are not firm in the soil, the stem is brittle, and can be easily broken off at the surface or a little below the surface of the soil. In an early stage of disease the stem when cut across shows a blue-green color; in a later stage it is brown. If the disease attacks a young plant, no head will form. There are also cases where the stem remains sound, and only the leaves are affected, those leaves having definitely outlined spots. In others the spots have coalesced, making a darkened mass of diseased tissue. A condition of hollow stem accompanied many of the diseased plants, but there were many plants without the disease which also had the hollow stem. This hollowness of the stem at the surface of the ground or just below it may have been due to unequal growth which followed a sudden check of rapid development or of regular growth. The effect of the hollow stem was varied: Some sound heads were produced; other plants were stunted; still others formed no heads. Where there was no discoloration in these hollow stems, no bacteria were found.

The different farms showed variable amounts of the lettuce disease. On one particular farm of 9 acres there were heavy losses. A patch of 3½ acres on this farm was examined very carefully by Dr. Joseph Rosenbaum, of the Bureau of Plant Industry, and by actual count 98 per cent of the plants were diseased (Pl. 30, A). Another farm of 17 acres suffered a loss of at least 60 per cent on a conservative estimate. On other farms visited the loss was much less, varying from 1 to 15 per cent.

The direct cause of the disease was thought to be a sudden drop in temperature which occurred the middle of February, when the mercury fell to 22° F. The plants were set out in December and January from perfectly healthy seed beds.

On examining into the different cultural and soil conditions on the various Beaufort farms several facts were brought to light. The soil throughout that locality is a sandy loam; the fertilizer used was made from marsh sedge, marsh mud, and leaf mold from swamps (live-oak leaves, etc.) composted with cattle, mule, and hog manure. To be in good condition for use, this compost should be allowed to decompose for two years because of the fibrous condition of the marsh sedge and the acidity of

the leaves in the compost. Those lettuce growers who had farms entirely free from disease did not use this compost under two years' aging (Pl. 30, B). Those who had had only a small amount of the disease had used it when about 1 year old or when they found that the grass had disintegrated. These last farms were protected by windbreaks and were not so exposed to the extreme cold; consequently little damage by the disease followed. The grower who lost 98 per cent on one plot had used compost only 7 or 8 months old and not thoroughly decomposed; even at the time of the lettuce harvest in April much of the marsh grass incorporated into the compost was sticking from the soil as stubble, and the plot, which suffered severely, was unprotected by windbreaks. This piece of land had never been planted to lettuce, and the year before a crop of cowpeas (*Vigna sinensis*) had been grown on it.

Cross-sections of stems in the blue-green stage and also in the brown stage were examined microscopically and bacteria were found swarming in the tissues. No fungi were present. Both the pith and the vascular region were involved. Moderately diseased plants were darkened only in patches in the vascular region of the stem. Bacteria were found in the brown spots of the leaves on plants where the stem was not diseased. The same organism was isolated both from the stem and from the leaves, and with it the disease was reproduced repeatedly by inoculations.

Some of the soil from two different farms in Beaufort County was obtained for tests in Washington, D. C. One of these farms was the one which suffered the 98 per cent loss. Lettuce plants in seedling stage, half-grown plants, and nearly mature plants were transplanted to pots containing this supposedly diseased soil. The plants were watched carefully for nearly a month, but no trace of the disease appeared.

Samples of soils from diseased and healthy fields were examined by Dr. Oswald Schreiner, Biochemist in Charge of Soil Fertility Investigations, Bureau of Plant Industry, but he could find no significant differences between the analyses of the diseased and healthy samples. It seems reasonable to suppose that the weakened state of the plants, owing to the extreme cold, put them in a condition in which bacterial organisms could readily gain access; and the continued weakened state of the plants after the cold spell passed allowed these organisms to use the plants as a good medium for their own growth and multiplication. There must have been considerable expansion and contraction of cells during the freeze and afterwards. This was shown by the frequent occurrence of splits in the stems at the surface and just below ground. This splitting or absence of splitting might account for the presence of bacteria in some stems and not in others. And the presence of the bacterial spots on the leaves where there was no stem infection might be the result of practically the same conditions following the expansion and contraction of cells of the leaves. The lower leaves and those nearest the soil were always the most spotted.

There is one point which stands out plainly in connection with the presence in the soil of active bacteria able to produce widespread infection in a lettuce crop in from three to six weeks. The worst infected field was composed of soil heavily impregnated with a compost still in the middle stages of decomposition (Pl. 30, A), and the plants were embedded in it so loosely because of the unrotted stubble that their roots were not well protected from the cold. The field was also unprotected by windbreaks.

In Beaufort County last year (1917) a freeze in February destroyed all the lettuce plants which had been set out in the early winter; but the second crop, planted in the early spring, matured without any evidence of this bacterial disease. A lettuce crop grown last spring in the area of the 98 per cent loss was entirely free from disease. The weather conditions remained favorable for growth during the season, and the plants had no setbacks. As the soil necessary for the quick growth of lettuce must be rich in decomposed organic matter, which likewise means one rich in soil organisms, it is difficult, in a late-fall- or winter-grown crop to eliminate the chance of these organisms getting into the plants should there be temperatures low enough to weaken the plants but not to kill them. Well-decomposed organic refuse, however, presumably has fewer active organisms of parasitic types, and the chances for infection are less should unfavorable weather conditions occur.

THE VIRGINIA LETTUCE DISEASE

An outbreak of disease on lettuce grown in soil rich in decomposing organic matter occurred also in the lettuce-growing region near Portsmouth, Va., early in November, 1916, following a heavy frost. At this time the heads were of good size, well filled out, and nearly ready to harvest. The disease was indicated by a spotting mostly on the outer leaves, where the spots frequently coalesced, making dark brown, almost black, widespread areas (Pl. 31). In some cases the browning and spotting ran along the midribs, but usually the infection was worse on the blades. In other cases the tip ends of the heart leaves were stained, but there was no definite spotting as in the outer leaves. The stems and roots were not infected. Many of the heads were cut open in the field, and the hearts were found to be all right, except for an occasional stain. Cross-sections of young spots were examined under the microscope, and bacteria were found in great numbers in the tissues. It seemed evident that they had entered the plants while these were in a weakened condition, and, getting a foothold, brought on the outbreak of disease in less than three weeks after the heavy frost. The lower and outer leaves, the parts most exposed to the cold, were the ones infected.

In the Portsmouth region the writer visited four lettuce farms where the disease was present, the loss varying from 10 to 40 per cent. The growers in this section use a commercial fertilizer, but also fertilize heavily with stable manure. This year they used fresh manure, the only

kind they could obtain, and it was all bought from the same source. The grower who had the highest percentage of disease had grown sorghum and cowpeas on his land and had plowed them under two to four weeks before the lettuce was planted there. The sorghum was not decomposed in November, so that very likely it made a splendid medium for the bacteria to live and thrive in. They would then be ready to attack the lettuce when it became weakened after the frost and could no longer resist their entrance.

There was one farm, which was inaccessible at the time of inspection because of heavy rains, on which the neighboring farmers said there was no disease. The growers claimed, too, that the same cultural and soil conditions obtained on this farm as on the others.

Two different bacteria were isolated from the Virginia lettuce plants, and even from the same plant but from different spots. One organism, which formed a distinct yellow growth on potato, proved to be identical with that isolated from the South Carolina lettuce (Pl. E, fig. 3). The other organism proved to be the same as one already described as causing a serious disease of lettuce in Louisiana in 1915.(*8*) This second organism (*Bacterium viridilividum*) forms, or may form, an evanescent blue-green growth on potato (Pl. E, 1). Both organisms were inoculated into lettuce plants, and both produced disease and later were reisolated.

THE LOUISIANA LETTUCE DISEASE

The Louisiana outbreak was at Nairn, Plaquemines County, La., during the winter of 1914–1915. About 200 acres of lettuce plants were infected, and the crop was almost a total loss. The plants were nearly mature when the infection overtook them. The outer leaves of the heads were the ones most affected, being either spotted or darkened throughout. The disease did not start in the stem or roots, for the center of the heads were sound and interior parts were rotted only when the disease spread in toward the center from the outer leaves. There had been excessive rainfall in this region for three months, and the unfavorable weather was thought to be the cause of the disease. The lowland plants were affected more severely than those on the high lands. A bacterium was isolated from the spots on the leaves, and by repeated inoculations with it into healthy lettuce plants it was proved to be the organism causing the disease.

As infection started in the outer leaves, it is reasonable to suppose that pathogenic organisms were washed up from the soil on the leaves, and when the plants became weakened through unfavorable weather conditions these organisms established themselves and the plants became diseased. The name "*Bacterium viridilividum*" was given this organism, and a report made of the disease by the writer (*8*). Illustrations of the Louisiana disease are included in this paper for comparison, as none were published in the earlier paper (Pl. E, 2; Pl. 32, A, B; Pl. 35, A, B).

THE KANSAS LETTUCE DISEASE

Another lettuce disease which has proved to be of bacterial origin came to the writer's attention through Mr. L. E. Melchers, of the Kansas Experiment Station. This was a disease of greenhouse lettuce (Pl. 33, A, B), and from the material submitted a bacterium not previously reported to be infectious to lettuce was obtained. Mr. Melchers's data on varietal susceptibility, appearance of the disease, etc., which were made in the greenhouse at Manhattan, Kans., are as follows:

The day temperatures in the greenhouse where the lettuce varieties were grown ranged as closely to 70° F. as possible, while the night temperatures ranged between 50 and 56° F. The disease first appeared about December 27, 1916, when most of the varieties were about half grown. The plantings had been made from October 19 to 26. Black Seeded Simpson (leaf lettuce) was the first to show the disease and this variety became badly affected. A second planting proved just as susceptible. The leaves in rosettes that are about half grown are perhaps the most susceptible. The Improved Hansen (head lettuce) also became badly attacked; it was second in susceptibility to Black Seeded Simpson. Big Boston (head lettuce) was about as susceptible as Improved Hansen. Early Curled Simpson (leaf lettuce) was less susceptible than the three mentioned varieties. Vaughan's All Season (head lettuce) only showed slight infection. Grand Rapids (leaf lettuce) seemed immune to attack, the disease did not appear on this variety.

The symptoms of this disease are quite striking. At first a slight marginal wilting takes place in more or less localized areas on leaves about the same age in the same whorl. The areas attacked in the leaf margins may vary from mere specks to areas two or three centimeters long and by coalescing, areas extending seven centimeters have been observed. The diseased areas scarcely ever extend more than three centimeters down the leaf, generally less than this. On the older leaves the most common sign is the wilting of the tips. The areas affected lop over and gradually become dry. The vascular tissues at this stage frequently show a distinct browning. In a few days the affected areas turn brown, tan, reddish, and sometimes black; the tissues become papery and dry in texture. This disease does not progress down the entire leaf, but ceases development after it extends a short way. It does not cause a rot or soft decay of lettuce but mars its appearance, so that it is not salable. Frequently the wilting symptoms do not appear until after a discoloration of the vascular system is noticed. Often brownish, water-soaked areas are seen and these tissues are turgid at the time. A speckled appearance is sometimes observed below the margins. This is caused by a slight discoloration of the vascular system in localized regions.

In a letter Mr. Melchers stated that he felt satisfied the infection comes from the soil and is carried to the plants by watering and by currents of air, that he suspected the disease was of bacterial origin, and that the organism gets its start by entering the younger leaves at the tips, where moisture is likely to remain for a longer time.

Spraying inoculations made with the organism isolated by the writer from the Manhattan plants proved that the organism enters the young leaves at the tips if they are kept moist. No wounding of the plants is necessary; poor ventilation is the only requisite after the plants are sprayed with water suspensions of young agar cultures.

Specimens of a lettuce disease which occurred in Hutchinson, Kans., were also sent to the writer by Mr. Melchers. This disease affected the Grand Rapids (loose-leaf) lettuce, the only variety at Manhattan not affected with the marginal disease. These plants had tiny irregular spots all over the blade and in the midrib; they were yellowish red in color, almost like rust spots (Pl. 34). In some places the spots coalesced. As in the Manhattan disease, bacteria were found swarming in the diseased places when cross-sections of those areas were examined microscopically. An organism was isolated from the Hutchinson plants which proved to be identical with the Manhattan organism. Inoculations proved this organism to be infectious. Besides the yellowish-red speckling, marginal infection also occurred. Successful inoculations were made into the Boston Head and Golden Queen varieties.

No further spread of the disease in this Hutchinson greenhouse was reported. The infection in all probability arose through an accident to the subirrigation system, for it was learned that in watering the plants through one of the tiles, the hose in some way worked out and threw the water for about half an hour over that part of the greenhouse where the disease occurred later. Because the disease did not occur on other, more susceptible varieties, and because the water from the disrupted irrigation system did not deluge those varieties, the accident is quite significant

It is the writer's opinion that the organisms dried up on the leaves of the Grand Rapids lettuce at Manhattan before they had a chance to enter them; consequently that variety did not become infected at the same time as the others in the same house. The very nature of loose-leaf varieties is such that there is better ventilation between the leaves; and if the young rosette at the center dries quickly after watering, there is little chance for the bacteria to get inside the leaf, since laboratory tests of this organism have shown that it is killed very readily by drying. In the case at Hutchinson where the disease occurred on the Grand Rapids variety, there was little chance for the drying out of any part of a leaf outside or toward the center of the head while the irrigation system was out of order. It is likely the bacteria were washed from the soil into the breathing pores of the leaves, for their presence later in irregular spots all through the blades shows that they took advantage of this condition, which was not confined to the margins of the moist center leaves.

ISOLATIONS AND INOCULATIONS WITH ORGANISM FROM THE SOUTH CAROLINA LETTUCE

The organism was isolated from the interior of the stem of the diseased South Carolina plants which showed the brown discoloration and also from the young spots on the leaves. The leaf portions were sterilized for one minute and the stems for two minutes in mercuric chlorid (1 : 1,000), washed in sterile water, mashed up in bouillon, and agar

plates were poured. The surface colonies appeared in from two to four days. They are at first a light-cream color, round, wet-shining, with fine surface markings. The margin is entire, with light and dark areas in an hourglass arrangement when viewed by transmitted light. When older, the colonies are yellow, without surface markings (Pl. 35, C).

Inoculations were made by spraying mature lettuce plants with pure cultures of the bacterium suspended in water (24- to 48-hour agar slants washed off in sterile water) and by pricking some of the leaves with a sterile needle. Those leaves of the older plants which were punctured became infected readily. Inoculations were made also by smearing the bacterial slime on the leaves and stem, and then puncturing the smeared places with a fine sterile needle (Pl. 36, A, B). Plants beginning to head or already headed became diseased readily, and those about to send up a seed stalk always showed the worst infection (Pl. 36, D, E). Young plants were only slightly affected, and usually recovered. Plants sprayed but not punctured rarely became infected. Repeatedly inoculations were made successfully; then the organism was reisolated; the reisolation colonies proved likewise to be infectious. The original colony kept growing on artificial media was still infectious a year after isolation.

The organism was inoculated into cabbage (*Brassica oleracea capitata*) in order to compare it with inoculations with *Bacterium campestre*. No infection followed with the lettuce organism, but *Bact. campestre* infected the cabbage readily. It was thought, too, that this lettuce organism might prove infectious to the heart of celery; therefore inoculations were made twice into young plants by spraying and by punctures, but with negative results. Nearly mature celery plants were treated in the same way with the same results.

DESCRIPTION OF THE SOUTH CAROLINA ORGANISM

The organism is a bacterium, a short rod with rounded ends, occurring singly or in pairs, occasionally in short chains. In stained host tissue the measurements of single rods vary from 0.62 to 1.04 μ long, and 0.42 to 0.83 μ wide (Pl. 41, A). Grown for one day on beef agar and stained with Loeffler's flagella stain, they vary from 0.62 to 1.24 μ long and 0.42 to 0.83 μ wide.

The organism is not actively motile; often in the sections of fresh tissue in which the bacteria occurred in numbers very little or no motion could be detected on microscopical examination. The motility was demonstrated better in young agar cultures. The flagella are polar, varying from one to several at each pole, but most commonly one at one pole. They were stained by Casares-Gil's flagella stain (Pl. 41, B).

Capsules were stained by Van Ermengem's flagella stain. The absence of spores was tested by staining and also by heating old live bouillon cultures. The tests were negative.

Pseudozoogloeæ occur and are composed of masses of short and long chains hanging together by a network of gelatinous threads. No long filaments or queer-shaped cells were noted. Swollen cells and others much reduced in size were noted in old cultures grown under low-temperature and high-temperature conditions, acid-media cultures, and cultures with sodium chlorid.

BEHAVIOR TOWARD STAINS

The organism stains readily and uniformly in the common anilin stains, such as gentian violet, methyl violet, dahlia, and carbol fuchsin. It is Gram-negative, and is not acid-fast.

CULTURAL CHARACTERS

Sterile potato cylinders proved to be a very good medium for this organism, the color of the bacterial slime being a bright yellow. Beef bouillon and litmus milk were favorable media for prolonged growth.

BEEF-AGAR PLATES.—The colonies on peptonized beef-agar plates (+15 Fuller's scale) are visible in 24 to 48 hours, room temperature 20° to 25° C., when poured from a young bouillon culture. They are at first a light-cream color, smooth, thin, round, edge entire with light and dark areas in a sort of hourglass arrangement. These areas disappear when colonies are 2 to 3 days old. Most of the colonies are cream color[1] throughout; some have blue areas or a ring of blue color with a cream center when viewed in transmitted light. When they are 4 to 5 days old, they are all a deep cream-yellow color, and from 3 to 6 mm. in diameter. Buried colonies are round or elliptical (Pl. 35, C).

AGAR STROKE.—In two days at 25° to 28° C. there is a moderate, cream-yellow growth, thin, flat, spreading, opaque, smooth, entire margin, viscid, yellowish in condensation water. Crystals abundant in three days. Growth remains moderate. Agar does not change color.

AGAR STAB.—There is very little growth in two days; in three days a fair amount of surface growth, faint filiform growth along line of puncture. Color of growth, honey-yellow. Crystals occur in agar just below the surface. In 14 days the color of the growth is old gold, and the crystals extend down into the agar near the puncture. In 30 days the growth is mustard color, and prismatic crystals occur throughout the agar.

BEEF BOUILLON.—Peptonized +15 beef bouillon is clouded faintly in 2 days at room temperature (25° to 28° C.). At 3 days most of the growth is at the surface until tube is agitated, and then the growth falls in tiny filmy flakes. No color change. In 8 to 10 days there is an interrupted pellicle, and the bouillon is yellowish, with a viscid sediment. In 41 days the pellicle is still incomplete, with long filaments hanging down in the medium. There is usually a yellow rim and a heavy viscid sediment in the bottom; the rest of the culture is usually clear and in color is old gold.

NEUTRAL BEEF BOUILLON.—In 3 days there is a faint growth at a temperature of 22° to 26° C., in 5 days a fair growth, and at 10 days a pellicle which sinks in long strands on handling the tube.

BOUILLON CONTAINING SODIUM CHLORID.—In 4 days there is slight growth in neutral beef bouillon containing 3 per cent of sodium chlorid. In 7 days there is a good growth. No growth occurs in the bouillon to which 4 per cent of sodium chlorid has been added.

[1] The colors mentioned in this paper are given according to Ridgway (RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., col. pl. Washington, D. C., 1912).

BOUILLON OVER CHLOROFORM.—Growth occurs, but is retarded. Only a slight clouding occurs in 7 to 8 days, and in 30 days the growth is still slight.

USCHINSKY'S SOLUTION.—The organism does not grow readily in this medium. There is a slight growth in 4 days. In 30 days it is still slight, with only a faint white clouding. This was repeated seven times. No growth occurred in three of the tests.

COHN'S SOLUTION.—The growth is very faint, and often does not occur. Out of eight tests definite growth occurred three times, faint growth twice, questionable twice, and once not at all. One infectious colony of the two used throughout this work might grow in one lot of media, while the other might not.

FERMI'S SOLUTION.—No growth occurred.

STERILE MILK.—In 5 days the medium is clear to a depth of only 3 mm. below the surface. In 7 to 9 days it is about half clear, with no acid coagulation, but with a heavy curdlike precipitate. There is a slow separation of curd and whey by 11 days. At 16 days there is no yellow color in the whey or the precipitate, but the bacterial growth at the surface is yellow. In 49 days very little curd is left, and this is present in little balls. The color of the whey has changed to yellow again, a light-orange yellow.

LITMUS MILK.—There is a slow reduction. The color changes in rings, a deep blue at the top, shading down to lilac litmus color at the bottom. In 5 days one-third of the medium from the top down is clear, and is darker blue, with a yellow bacterial precipitate. In 7 days a curd has formed; there are still three shades in the medium, anthracene-purple at the top, shading to a brownish at the bottom. At 18 days the purple color has disappeared, and the entire medium is a light brown; the curd is in suspension. After a month the medium is light brown throughout, except at the very surface, where it is purple. There is a viscid mixture of curd and bacteria through half the medium, numerous balls of white curd floating in this viscid mixture.

NUTRIENT GELATIN.—The colonies are slow in appearing on peptone gelatin (+10) plates at 12° to 15° C. In two tests made the colonies did not appear before 7 to 9 days. Even when the plates are not thickly sown, the colonies do not develop a diameter larger than 4 mm. They are yellow, round, shining, and thicker than beef-agar colonies. Buried colonies are both round and oval. Liquefaction begins when the colonies are 3 days old, in little cups around them, continuing slowly. When they are 16 days old, the gelatin of the thinly sown plates has not entirely liquefied.

The stab cultures liquefy slowly also at a temperature of 12° to 15° C. In 2 days there is slight growth on the surface and along the line of puncture, but no liquefaction. In 9 days there is a slight crateriform liquefaction, and in 12 days the liquefaction has reached almost across the surface of the gelatin. In 30 days 1.5 cm. of the medium are liquefied, in 40 days one-half, and in 57 days all except one-sixth at the bottom of the tube.

STEAMED POTATO CYLINDERS.—There is abundant growth in 2 days at a temperature of 25° C. The growth is smooth, thick, viscid, shining; the color is empire-yellow (Pl. E). In 14 days the growth is a dark olive-buff, and the medium has changed to a grayish brown.

There is a feeble diastasic action on the starch.

OTHER CULTURAL FEATURES OF THE ORGANISM

INDOL.—There is slight production of indol in 1 per cent peptone-water cultures 10 days old. It is still slight when the cultures are 16 to 20 days old.

NITRATES.—Nitrates are not reduced. Tests were made when nitrate bouillon cultures were 7 and 17 days old.

AMMONIA PRODUCTION.—Moderate.

HYDROGEN SULPHID.—Hydrogen sulphid is produced. Cultures of beef agar, beef bouillon, milk, and potato cylinders were tested by hanging lead-acetate paper in

the tubes where the transfers were made. The paper became well blackened in every case.

TOLERATION OF ACIDS

Tests were made with tartaric, malic, and citric acids, and it was found that the organism is most sensitive to the presence of small quantities of citric acid. There was a good growth in neutral beef bouillon with 0.1 per cent of tartaric acid, which titrated +23 on Fuller's scale, but no growth at all where 0.2 per cent of tartaric acid was added. There was good growth also in neutral beef bouillon to which 0.1 per cent of malic acid was added, which titrated +25 on Fuller's scale. There was no growth in the bouillon to which 0.2 per cent of malic acid was added. Three tests were made with neutral beef bouillon to which 0.1 per cent of citric acid was added, but growth occurred only once, in which the medium titrated +17. The negative tests titrated a little higher.

TOLERATION OF SODIUM HYDROXID

The organism tolerates sodium hydroxid to −25 on Fuller's scale. Tests were made in beef bouillon containing sodium hydroxid titrating −20, −25, −30, −35, and −40. In 2 days there was a slight clouding in −20 and in −25, but none in −30, −35, or −40.

TEMPERATURE RELATIONS

Thermal death point.—When transfers are made from a well-clouded bouillon culture of 24 hours and kept at 52° C. in a water bath for 10 minutes, no growth occurs. This test was repeated many times. Sometimes growth occurred at 51°. The thermal death point lies, therefore, between 51° and 52°.

Maximum temperature.—The maximum temperature for growth is 35° C.

Minimum temperature.—The minimum temperature for growth is below 0° C.

Optimum temperature.—The optimum temperature is 26° to 28° C.

Beef agar and bouillon cultures were used for the three preceding temperature tests.

Gas formation.—The organism is aerobic and does not form gas. It was tested in fermentation tubes in the presence of each of the following carbon compounds: Glycerin, dextrose, lactose, saccharose, maltose, and mannit, 1 per cent of these being added to a 1 per cent water solution of Witte's peptone. No gas formed in any of the tubes, and no growth took place in the closed arm of the tubes. However, growth occurred in the open end of each tube. In the test for acid and alkaline reactions with neutral litmus paper all showed alkaline reactions.

FURTHER TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. Tests were made by placing agar and bouillon transfers in a specially devised jar from which the oxygen was removed in the following way: 40 gm. of pyrogallic acid were dissolved in potassium hydroxid (35 gm. to 350 c. c. of water), and this mixture was placed uncovered in a bottle in the jar with the cultures. The top of the jar was covered; then another cover inserted in a bed of mercury was placed over the whole. The experiment was watched carefully, yet no growth could be detected until the cultures were removed at the end of two weeks, when one of the bouillon cultures was found to have developed a few threads of filamentous growth extending from the surface into the medium, but no clouding occurred. No growth occurred in the stab cultures, a trace of growth in one tube not being considered significant. The control cultures showed good growth in one day. After removal from the jar growth took place in the bouillon cultures, but there was none in the agar.

RELATION TO LIGHT

The organism is not very sensitive to sunlight. Thinly sown agar plates were exposed bottom side up on cracked ice, one side of the plate being covered with black paper. Midday on bright sunny days in early winter was taken for the test. The temperature of the ice bag was 8° to 10° C. A 40-minute exposure did not kill the organism, and in some tests even a few colonies appeared after 50 minutes' exposure; but no colonies appeared on those plates exposed for 60 minutes.

RELATION TO MOISTURE

The organism is not killed very readily by drying. Drops of a 1-day-old bouillon culture were transferred to sterile cover glasses in a petri dish, and the dish was placed in the dark. The temperature of the room during the days of this test was 25° to 30° C. When kept for two days, and dropped in tubes of bouillon, growth occurred; but no growth occurred in those tubes which received covers on which the organism had been drying for three days.

VITALITY IN CULTURE MEDIA

This bacterium lives for more than a year in liquid culture media when cultures are kept in the refrigerator at temperatures of 12° to 15° C., and do not evaporate readily. At room temperatures (20° to 25°) it lives from two to three months. Milk and litmus milk are the most favorable media for continued growth at these temperatures. In two months the organism is dead in bouillon and on potato cylinders.

LOSS OF VIRULENCE

No loss of virulence was noticed when inoculations were made within eight months to a year after isolation.

GROUP NUMBER

According to the descriptive chart of the Society of American Bacteriologists, the group number is 211.3332523.

The name "*Bacterium vitians*, n. sp.," is suggested for this organism.

BRIEF TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium vitians, n. sp.

A short motile rod with rounded ends, flagella bipolar, but usually one at one pole; capsules, pseudozoogloeæ, no spores, involution forms rare and few types, aerobic; agar colonies, light-cream color, smooth, thin, round, light and dark areas in an hourglass arrangement when young; when older, shading disappears, and all are cream-yellow. Growth on potato cylinders is abundant, bright yellow; produces alkaline reaction in litmus milk, with a gradual separation of the whey from the curd, curd partly digested; liquefies gelatin slowly; produces ammonia, hydrogen sulphid, indol (slight); does not reduce nitrates; feeble diastasic action on potato starch; grows in Uschinsky's solution; grows feebly or not at all in Cohn's solution; thermal death point 51° to 52° C. Maximum temperature for growth 35° C., minimum below 0° C., optimum 26° to 28° C. Vitality two months to over a year in liquid media, depending on temperature and evaporation. Is Gram negative, and is not acid-fast; stains readily with basic anilin dyes. Not killed very readily by drying, not very sensitive to sunlight; slight toleration of acids and alkalies (tolerates tartaric in neutral beef bouillon to +23 Fuller's scale, malic +25 Fuller's scale, citric +17; tolerates sodium hydroxid in beef bouillon to −25); retains its virulence over one year.

ISOLATIONS AND INOCULATIONS WITH ORGANISMS FROM VIRGINIA LETTUCE

Two organisms were isolated from the spots in the diseased plants from the lettuce-growing sections along Hampton Roads, Virginia: one (*8*) the Louisiana organism, *Bacterium viridilividum* (Pl. 35, D), and the other the South Carolina organism, *Bacterium vitians*. Isolations were made from plants from three different farms, and whatever skepticism there might have been at first because of the presence of two distinct pathogenic organisms was dispelled when the two familiar colonies persisted in appearing on the plates.

The isolations of *Bact. viridilividum* produced spotting of the leaves when inoculated into greenhouse plants (Pl. 37, A). The isolation of *Bact. vitians* also produced spotting and rotting of leaves when inoculated into greenhouse plants (Pl. 38), and likewise the typical stem disease when inoculated into the stems (Pl. 37, B). There was no natural infection of the stems in the diseased lettuce from the Virginia fields, but the inoculations in the stem were made to prove the full pathogenicity of the organism as compared with that isolated from the

South Carolina plants. In South Carolina the stem infection was more prevalent by far than the leaf spotting alone. *Bact. viridilividum* from the Virginia plants became blue-green on sterile potato cylinders (Pl. E, 1) the same as *Bact. viridilividum* from Louisiana (Pl. E, 2), but like the Louisiana isolation, the color is fleeting, and frequently there are infectious colonies which will not produce the blue-green color on potato, but which agree in other cultural features.

Morphological and cultural tests were made with *Bact. vitians* from the two sources, South Carolina and Virginia, and no doubt remains as to their identity.

ISOLATIONS AND INOCULATIONS WITH ORGANISM FROM THE KANSAS LETTUCE

ISOLATION OF THE ORGANISM

Pieces of the browned marginal areas from the diseased material from Manhattan, Kans., and of the small irregular reddish spots from the material from Hutchinson were used for isolating. The pieces were immersed in mercuric chlorid (1 : 1,000), one test for 2 minutes and another for 3 minutes, washed in sterile water, and mashed up in bouillon. Surface colonies appeared in two days, thin, bluish white, round, shining, some slightly convoluted, most with a smooth surface. The color changes to cream, then yellowish, and the agar becomes a brilliant green. The colonies range from 2 to 7 mm. in diameter when several days old.

INOCULATIONS

Inoculations with the organism isolated from the Kansas lettuce were made by spraying water suspensions of young agar cultures on young and half-grown lettuce plants. No wounding was necessary to produce infection. The margins of the inner whorl of leaves became dark brown, almost black, in 24 to 48 hours; the outer leaves were not infected. At first these brown margins were soft, but in a few days they became dry and papery, with a brown discoloration extending farther in the veins and veinlets. This condition had been noted on the infected leaves received from Kansas (Pl. 39, A, B). The infected margins were from 0.5 to 1.5 cm. in width, rarely wider. A very tiny curled-up leaf might be entirely browned. Some of the infected places, but not all, first showed as little reddish and brownish spots, and the veins showed darkening before the parenchyma.

Inoculations were made on plants growing in the open bed and also in pots, which were placed in infection cages where there was plenty of moisture (Pl. 40, A, B). Occasionally there would be a plant which resisted infection in the open bed, but scarcely ever one in the infection cage. The temperature of the greenhouse did not seem to have so

much effect on the results of the inoculation experiments as the ventilation and moisture.

The inner whorl of leaves was the part of the plant usually infected. No mature closed heads were inoculated, but occasionally in infection-cage experiments some of the older leaves had numerous red speckled areas, which, on examination, proved to be filled with bacteria.

One of the inoculation tests was made in two greenhouses during the winter when the temperature of one was 8 to 10 degrees lower than that of the other. The plants were placed in infection cages, where there would be little ventilation and high humidity. The disease took readily in both houses. This test was followed up in the summer, when both houses were practically of the same temperature. The plants were grown in open beds, those in one house being set farther apart and kept better ventilated than the other one. The plants of both houses were inoculated by spraying them with the same cultures. The infection produced in the well-ventilated house was almost negligible, while the usual blackened margins of the inner rosette of leaves occurred on the close-set plants in the poorly ventilated greenhouse. The organism was reisolated from the diseased margins, and on inoculating with the colonies so obtained, the disease was again produced.

This disease of lettuce need not be confused with the browning of margins of lettuce leaves due to tipburn, or sunscald, for the brown of tipburn is a much lighter color.

The hearts of young and old celery plants were inoculated with the Kansas organism by spraying, also by smearing the bacterial slime on the leaves and then puncturing them. No infection followed on either young or old plants.

DESCRIPTION OF THE KANSAS ORGANISM

The organism is a bacterium motile by means of polar flagella, one or two at each pole, a few noted with three at a pole Casares-Gil's flagella stain, (Pl. 41, E). It is a short rod rounded at the ends, occurring in short chains or singly. Stained with carbol fuchsin in the leaf it is 0.83 to 1.66 μ long and 0.83 to 1.25 μ wide, the majority being 1.45 μ long and 0.83 μ wide. Grown on beef agar for 24 hours and stained with gentian-violet, it is 0.83 to 1.87 μ long and 0.42 to 0.83 μ wide. Stained with carbol fuchsin, same age, it is 1.25 to 2.08 μ long and 0.42 to 0.83 μ wide.

Capsules were stained by Ribbert's capsule stain (Pl. 41, D).

Endospores are not produced. Tests were made by boiling several liquid cultures of different ages for 3 minutes; also heating others to 80° C. for 20 minutes. Transfers were made in each case before and after boiling. Before heating and boiling all cultures were alive, as growth took place in the transfers, but none took place in transfers made from cultures after they had been boiled or heated. The organism forms

pseudozoogloeæ, grows in clumps very quickly in some of the liquid media—for example, in beef bouillon—and when first isolated it produces a very offensive odor. About 4 or 5 months after isolation, the odor was considerably less, and in 8 months none of it could be detected.

BEHAVIOR TOWARD STAINS

The organism stains very readily in methyl violet, carbol fuchsin, safranin, and dahlia. It is Gram-negative, and is not acid-fast.

CULTURAL CHARACTERS

This organism grows readily in most of the media, and is a much more rapid grower than either *Bact. vitians* or *Bact. viridilividum*. It belongs to the green fluorescent group. Beef bouillon (+15) is a very successful medium. In fact, growth took place too rapidly in this medium for many tests where a thinly clouded culture was needed, and low temperatures had to be used as soon as the transfer was made, or an acid bouillon or a 5 per cent sodium-chlorid bouillon used to delay the too rapid growth.

BEEF-AGAR PLATES.—Colonies appear in from 24 to 48 hours when poured from a young bouillon culture. The temperature of the room may vary from 22° to 30° C. At first the surface colonies are a faint bluish white, then cream color, and later a yellowish color; they vary in size from 3 to 7 mm. in diameter, are round, smooth, occasionally convoluted, thin; at first there are fine surface markings which disappear as the colonies get older (Pl. 35, E). The agar becomes yellow-green.

AGAR STROKE.—The growth in 2 days is thin, spreading, yellow. The agar just below the surface is a viridine green. The surface of growth is rather finely papillate than smooth. In 10 days all the agar is colored Javel green; the growth is abundant, glistening, viscid.

AGAR STAB.—The surface growth is rapid, but growth is feeble along the stab. At 2 days it is cream-colored with no discoloration of agar. In 7 days growth is yellow and nearly covers the surface; the agar is viridine green just below the growth at the surface and along the stab. Crystals appear below the surface.

BEEF BOUILLON.—Peptonized +15 beef bouillon is clouded very readily in 18 to 24 hours at temperatures of 20° to 30° C. At temperatures of 5° to 11° it is thinly clouded in 24 hours. Usually a pellicle which breaks up easily has developed in 3 days; the bouillon is viridine green at the surface and for about 2 cm. down. In 6 days the upper part of the medium is apple-green, and besides the pellicle there is a white precipitate. In 30 days there is a heavy viscid growth at the bottom of the tube; the bouillon is clear; and the color is olive-ocher.

BOUILLON OVER CHLOROFORM.—Chloroform does not retard the growth. Clouding takes place in 24 hours, and a heavy growth follows.

Bouillon containing sodium chlorid.—There is good growth at once in neutral beef bouillon containing 3 per cent of sodium chlorid, a slight retardation but heavy growth in that containing 5 per cent, only a fair amount of growth in the bouillon containing 6 per cent, and none at all in that containing 8 per cent.

Gelatin plates.—Colonies are up in 4 days on +10 beef-peptone gelatin plates at 11° to 15° C.; are 1 mm. in diameter, a deep-cream color, bluish in transmitted light, with margins slightly indented. Liquefaction (cup-shaped) begins when colonies are 2 days old. No color change occurs at this age. When colonies are 5 days old, they are 2 to 4 mm. in diameter and the gelatin is greened around them for some distance. This color is mineral-green. At 15 days the liquefaction is still cup-shaped around colonies, but the green color has spread through the entire gelatin. At 25 days the gelatin is not all liquefied.

Gelatin stab.—In beef-peptone gelatin +10 stabs liquefaction begins in from 2 to 4 days in a crateriform way at a temperature of 11° to 15° C. Growth is good at the surface, feeble along the stab; there is a faint green color at the surface. In 6 days gelatin is liquefied three-fourths across the surface of the stab, the color just below being viridine green. In 15 days the surface of the gelatin is liquefied straight across for a depth of 1 cm. and the color, which extends halfway down the tube, is yellow-green, a brighter green than that of the plates.

Uschinsky's solution.—There is heavy clouding in 24 hours. In 3 days the upper half of the medium is greened. In 5 days there is a heavy pellicle, and the medium throughout has become Veronese-green.

Cohn's solution.—The organism does not grow in Cohn's solution.

Fermi's solution.—No growth.

Sterile milk.—In 3 days about one-third of the medium is cleared and is a sea-foam-green color; the rest is a soft curd. In 20 days the milk is all cleared with curd in suspension. The color is citron-green. In 47 days the milk is a darker color, lime-green. The curd is in suspension in bottom of tube; the rest of the liquid is clear.

Litmus Milk.—In 2 days the color of the medium has begun to change in rings, a turbid reddish color at the top for 4 mm., then 2 cm. of a lighter shade below, and next the ring of lilac-litmus color. A pellicle is on the surface; there is no coagulation. In 4 days none of the original lilac-litmus color is left; the medium is clear and half of it is reddish brown (dark vinaceous drab); the rest is a lighter color. At 7 days the upper half of the medium has more red color in it—is dark mineral-red. The rest of the milk is a reddish-tan color. There is a heavy pellicle and a soft curd. In 25 days the medium has changed to a dark-blue shade, is blue-violet-black.

Steamed potato cylinders.—There is a thin watery growth covering the surface of the cylinder in one day. Plate E, 5, gives the appearance at the end of the second day. In 4 days this growth is a pinkish-

tan color, but is still thin, and the medium is unchanged. In 13 to 16 days (Pl. E, 6) the color of the growth is warm-buff and the potato has darkened slightly. In 30 days there is no further change. There is feeble diastasic action on potato starch.

OTHER CULTURAL FEATURES OF THE ORGANISM

INDOL.—No indol is produced.

NITRATES.—There is a good reduction of nitrates. Tests were made with nitrate bouillon cultures in which the organism grew very well. One c. c. potato-starch solution was added to each culture; then one c. c. of a fresh potassium-iodid solution (1:250), after which five drops of dilute sulphuric acid (2:1) were added. A dark-blue color, indicating reduction, followed immediately.

HYDROGEN SULPHID.—No hydrogen sulphid was detected.

AMMONIA.—The organism produces ammonia. Cultures of bouillon agar, and Uschinsky's solution (2 to 6 weeks old) were tested with Nessler's solution. Strips of filter paper were moistened with the solution and suspended in the tubes to be tested. A brownish-red color appeared on the filter paper immediately, indicating the presence of ammonia.

TOLERATION OF ACIDS.—There is a moderate toleration of citric, malic, and oxalic acids. In the tests these acids were added to neutral beef bouillon.

A good growth occurred in two days in the bouillon containing citric acid titrating +37 on Fuller's scale, but no growth occurred in that titrating +39.

With malic acid there was a good growth in four days in the solution titrating +38, but none occurred in that titrating +40.

With oxalic acid there was a good clouding in +37 in six days, but no growth in +40.

TOLERATION OF SODIUM HYDROXID.—The toleration of sodium hydroxid by this bacterium is moderate. There is heavy clouding and pellicle in −20 beef buillon, fair clouding in −25, and faint clouding in −30 in two days. No growth occurs in −40.

GAS FORMATION.—The organism is aerobic, and does not form gas. Tests were made in fermentation tubes with water containing 1 per cent of Witte's peptone to which was added 1 per cent of each of the following carbon compounds: Dextrose, lactose, saccharose, maltose, glycerin, and mannit. Growth occurred in the open end of the tubes, but none took place in the closed end and no gas was produced. Dextrose and saccharose gave an acid test with litmus after the organism had been growing in the tubes for six weeks. Glycerin, maltose, mannit, and lactose gave an alkaline test.

FURTHER TEST FOR ANAEROBISM

The organism will not grow in an atmosphere deprived of oxygen. A special flask from which the oxygen had been absorbed by a mixture of pyrogallic acid and potassium hydroxid was used for the test (described on p. 374). Transfers of the organism were made to +15 bouillon and beef-agar stabs and placed in the jar. At the end of two weeks, when they were removed, there was a mere trace of growth in the stabs, and there was a thin pellicle on the top of one of the bouillon cultures. But there was no clouding and no green color so characteristic of this organism. The controls showed good growth in one day. The organism is a rapid grower, and it is likely that the oxygen in the culture tubes was not absorbed promptly enough to exclude all growth. Seven days after removal from the jar the bouillon cultures were clouded, but no growth had taken place in the agar.

TEMPERATURE RELATIONS

THERMAL DEATH POINT.—The thermal death point lies between 52° and 53° C. when transfers are made from a thinly clouded culture which does not contain clumps of bacteria and they are kept in the water bath for 10 minutes. If an 18 to 24 hour old +15 bouillon culture which is densely clouded is used there will be growth when the transfers are subjected to 55° and 56° C. for 10 minutes. This is because of the tiny masses of bacteria which hold together in clumps, the inner ones of which are somewhat protected.

MAXIMUM TEMPERATURE.—The maximum temperature for growth is 38° C.

MINIMUM TEMPERATURE.—The minimum temperature for growth is below 0° C.

OPTIMUM TEMPERATURE.—The optimum temperature for growth is 25° to 26° C.

The medium used for these three preceding temperature tests was +15 peptone-beef bouillon.

RELATION TO LIGHT

The organism is not particularly sensitive to sunlight. The different sets of plates for this test were poured from bouillon cultures that were not heavily clouded. The plates were exposed to bright sunlight at noonday in June and July, one-half of each plate being covered with carbon paper and placed, bottom side up, on sacks of cracked ice, the temperature of the bag being 8° to 14° C. No colonies appeared on the uncovered side of the plates exposed for 40 minutes while from 50 to 70 colonies appeared on the covered sides. In four separate tests colonies appeared twice on the exposed side of 35-minute plates, and twice none appeared. On 30-minute plates 1 to 10 colonies appeared on the exposed sides

to over 50 on the covered sides. On 25-minute plates from 3 to 5 colonies appeared on the exposed sides, while more than 50 appeared on the covered sides. Many colonies appeared on the exposed sides of 15 and 20 minute plates.

RELATION TO MOISTURE

The organism is killed readily by drying. When a +15 bouillon transfer is kept in the refrigerator for one day at 11° to 12° C., there is clouding but no heavy growth. If transfers of drops are made to sterile cover glasses from such a culture and the cover glasses kept in the dark at 25° to 27°, the bacteria die in five hours but are still alive at three hours. The test was made by dropping them in tubes of beef bouillon after those intervals had elapsed.

If a 1-day-old heavily clouded +15 bouillon culture, which contains the clumps of bacteria is used, drying does not take place so readily, and cover glasses in the dark at 24° will still have live bacteria on them after drying for six days.

VITALITY IN CULTURE MEDIA

The organism lives for 5 months in beef-agar stabs and more than six months in beef bouillon and sterile milk when kept at room temperatures varying from 24° to 30° C. If evaporation is such that the cultures dry down, they will die before this time has elapsed Cultures kept in the refrigerator will live from 9 to 10 months.

LOSS OF VIRULENCE

The organism is still virulent at the time of writing, more than a year after isolation.

GROUP NUMBER

According to the descriptive chart of the Society of American Bacteriologists, the group number is 211.2323123.

The name "*Bacterium marginale*, n. sp." is suggested.

BRIEF TECHNICAL DESCRIPTION OF THE ORGANISM

Bacterium marginale, n. sp.

It is a short rod with rounded ends; flagella 1 to 3 bipolar, capsules; pseudozoogloeæ; no spores; few involution forms noted; aerobic; agar colonies cream-colored when young, yellow when mature and the agar a brilliant green; clouds bouillon very heavily in 24 hours at 20° to 30° C., and in six days the medium is apple-green; growth on potato cylinders is scanty and dirty cream-colored (Pl. E, 5, 6); later it is a warm-buff. The potato darkens slowly; the diastasic action is feeble; liquefies gelatin slowly; produces ammonia; fluorescence green; reduces nitrates; does not produce indol nor hydrogen sulphid; grows in Uschinsky's but not in Cohn's or Fermi's solution; optimum temperature 25° to 26°; maximum 38°; minimum below 0°. Thermal death point 52° to 53° (under conditions stated); vitality at room

temperature six months in liquid media; stains readily with basic anilin dyes; is Gram-negative; not acid-fast; not very sensitive to sodium chlorid (tolerates 6 + per cent); moderate toleration of acids and alkalies (tolerates oxalic to + 37 on Fuller's scale; malic + 38; citric + 37; tolerates sodium hydroxid in beef bouillon to −30 Fuller's scale); is killed readily by drying; not very sensitive to sunlight; retains its virulence for more than one year.

CONTROL OF LETTUCE DISEASES

THE SOUTH CAROLINA AND VIRGINIA DISEASE

So far as known, the *Bacterium vitians* gets into the field lettuce only when it is in a weakened state owing to sudden cold weather which is not cold enough to kill the plants. The treatment recommended is the use of thoroughly decomposed green manure and well-seasoned stable manure in which tissue-disintegrating bacteria have practically finished their work. The bacteria then present in the soil are not active and the plant, though weakened by sudden severe cold, may regain its stability and be able to resist their entrance.

The use of satisfactory windbreaks is obvious.

THE KANSAS DISEASE

As *Bacterium marginale* is a soil organism also, care should be taken in watering the plants in the greenhouses that the roots only of lettuce are watered. Soil should not be washed up nor spattered on the leaves. Subirrigation is a safeguard.

Good ventilation will almost, if not entirely, prevent the disease.

SUMMARY

Two new bacterial diseases of lettuce are described in this paper. One occurred in South Carolina and in Virginia the same year both on winter and late fall crops grown out of doors. The other is a disease of greenhouse-grown plants in Kansas.

The South Carolina disease occurred in the stems and roots and less frequently on the leaves, following a sudden drop in temperature in February. The Virginia disease occurred on the leaves only and followed a heavy frost in October. An infectious organism identical with the South Carolina bacterium was isolated.

Inoculations were made with the bacterium isolated from the South Carolina and Virginia lettuce, and this organism from both sources proved to be infectious to both stem and leaves of lettuce. The name "*Bacterium vitians*" is suggested for this organism.

Besides this organism another was present in the Virginia lettuce. This was recognized as *Bacterium viridilividum*, an organism known previously to produce a lettuce disease. This colony also proved to be infectious.

It appears that both of these organisms are present and active in soil in which there is abundant green manure or stable manure which has not been thoroughly decomposed. If conditions are such that the plant keeps up a steady growth and is not checked, these bacteria do not enter. When conditions are such that the plant is weakened or growth checked, an entrance is gained and disease follows.

The marginal disease of greenhouse lettuce reported from Kansas is also caused by a soil bacterium. The name "*Bacterium marginale*" is suggested. The margins of the inner whorl of leaves of immature plants are most frequently infected, but the entire leaf can be speckled or spotted by infection, which depends on defective greenhouse conditions. Subirrigation and proper ventilation will prevent this disease.

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PLATE E

1.—*Bacterium viridilividum*, second organism isolated from Virginia lettuce: Appearance of the growth on potato at the end of 2 days.

2.—*Bacterium viridilividum*, original organism from Louisiana: Appearance of the growth on potato at the end of 2 days. Later, the upper part of the fluid becomes buff colored.

3.—*Bacterium vitians*, first organism isolated from Virginia lettuce: Appearance of the growth on potato at the end of 2 days.

4.—*Bacterium vitians*, isolated from South Carolina lettuce: Appearance of the growth on potato at the end of 3 days.

5.—*Bacterium marginale*, isolated from Kansas lettuce: Appearance of the growth on potato at the end of 2 days.

6.—*Bacterium marginale*, isolated from Kansas lettuce: Appearance of the growth on potato at the end of 13 days.

Painted by Mr. James F. Brewer.

1

2

3

4

5

6

PLATE 29[1]

Bacterium vitians:

A.—Lettuce from Beaufort, S. C., showing stems blackened by the disease. Photographed by Dr. J. Rosenbaum.

B.—Lettuce leaves from South Carolina, showing spotted-leaf type of the disease. In many cases the stems were sound.

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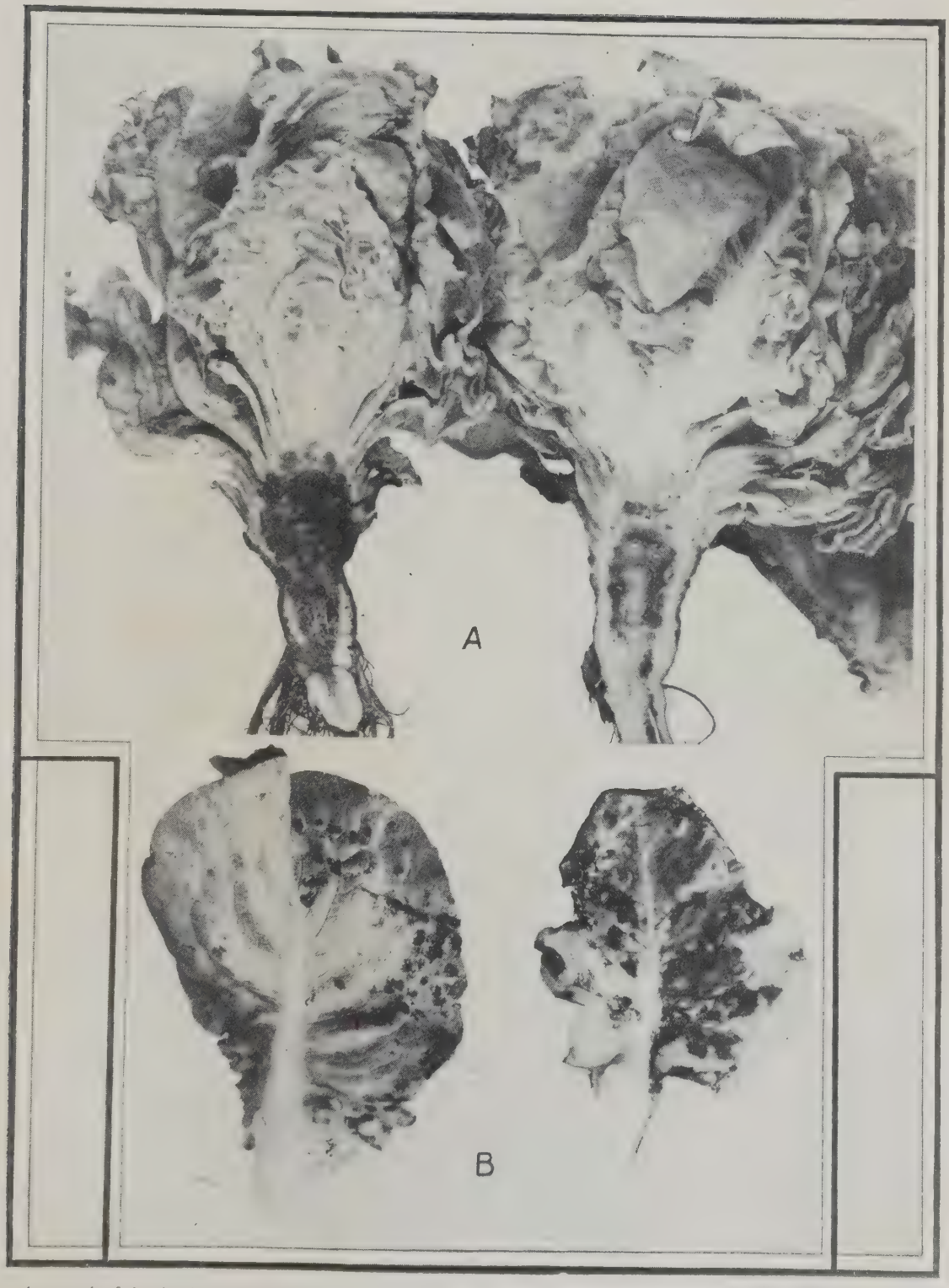
[1] Photographs and photomicrographs reproduced in Plates 29 to 41 were made by Mr. James F. Brewer, except as otherwise stated.


A
B

A
B

PLATE 30

Bacterium vitians:

A.—A field of 3½ acres of diseased lettuce at Beaufort, S. C. By actual count there are two good plants in a hundred. Photographed by Dr. J. Rosenbaum.

B.—A field of healthy lettuce at Beaufort, S. C. This field was planted with seed from the same lot as that sown in the field shown in A, but the land received different treatment previous to planting and afterward.

PLATE 31

Two badly diseased leaves of Virginia lettuce, from which both *Bacterium viridilividum* and the South Carolina yellow organism *Bact. vitians* were isolated.

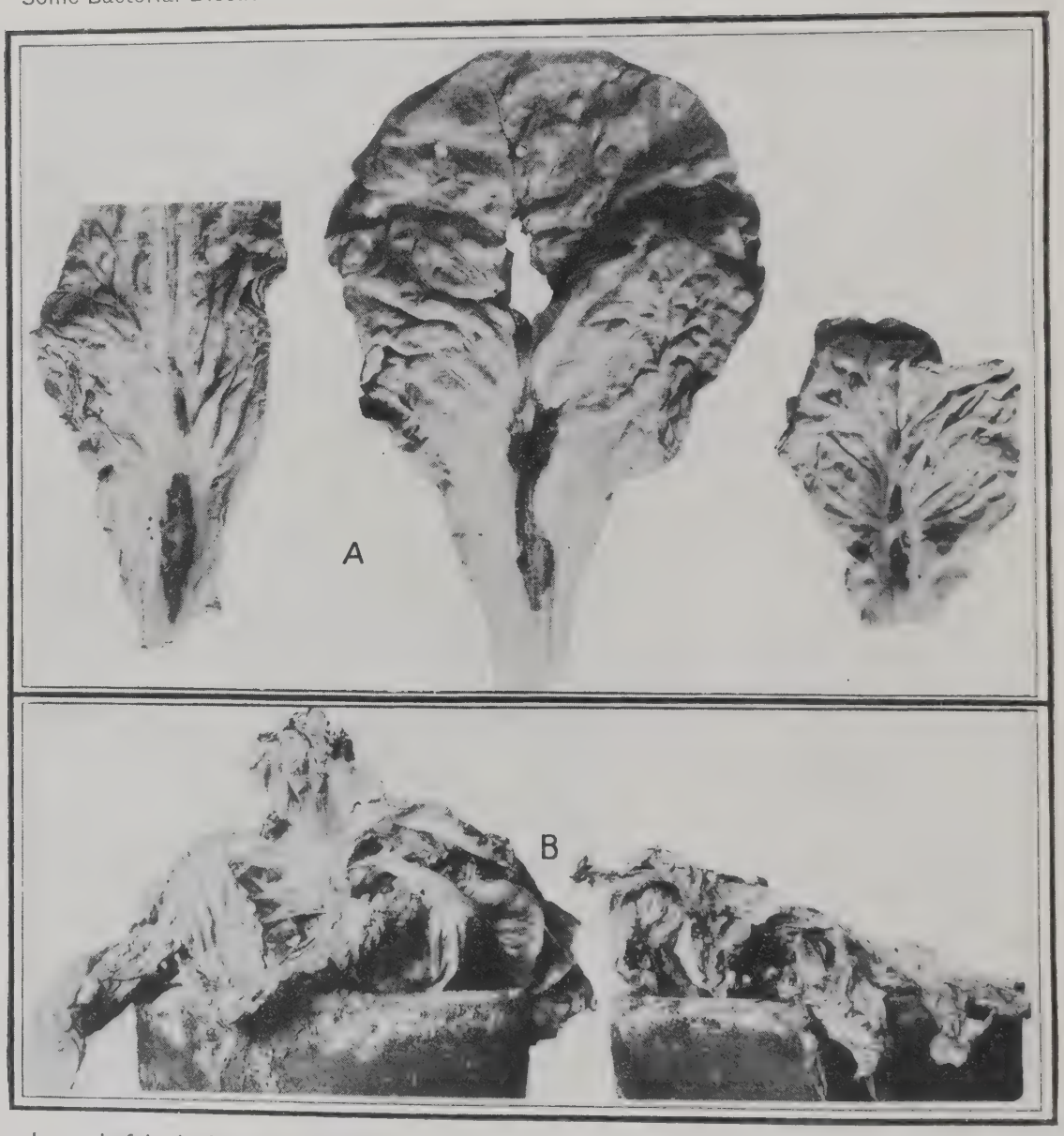

A
B

PLATE 32

Bacterium viridilividum, the cause of the Louisiana lettuce disease:

A.—Three leaves of lettuce inoculated by needle pricks on February 15, 1915. Photographed on February 17, 1915. The tissues are blackened, and decay is progressing rapidly.

B.—Two pots of lettuce inoculated by spraying on February 19, 1915. Photographed on March 9, 1915.

PLATE 33

Bacterium marginale, the cause of the Kansas lettuce disease:

A.—A head of diseased lettuce from Manhattan, Kans. Photographed by Mr. L. E. Melchers.

B.—Single leaves of Manhattan lettuce, showing the effect of the marginal disease. Photographed by Mr. L. E. Melchers.

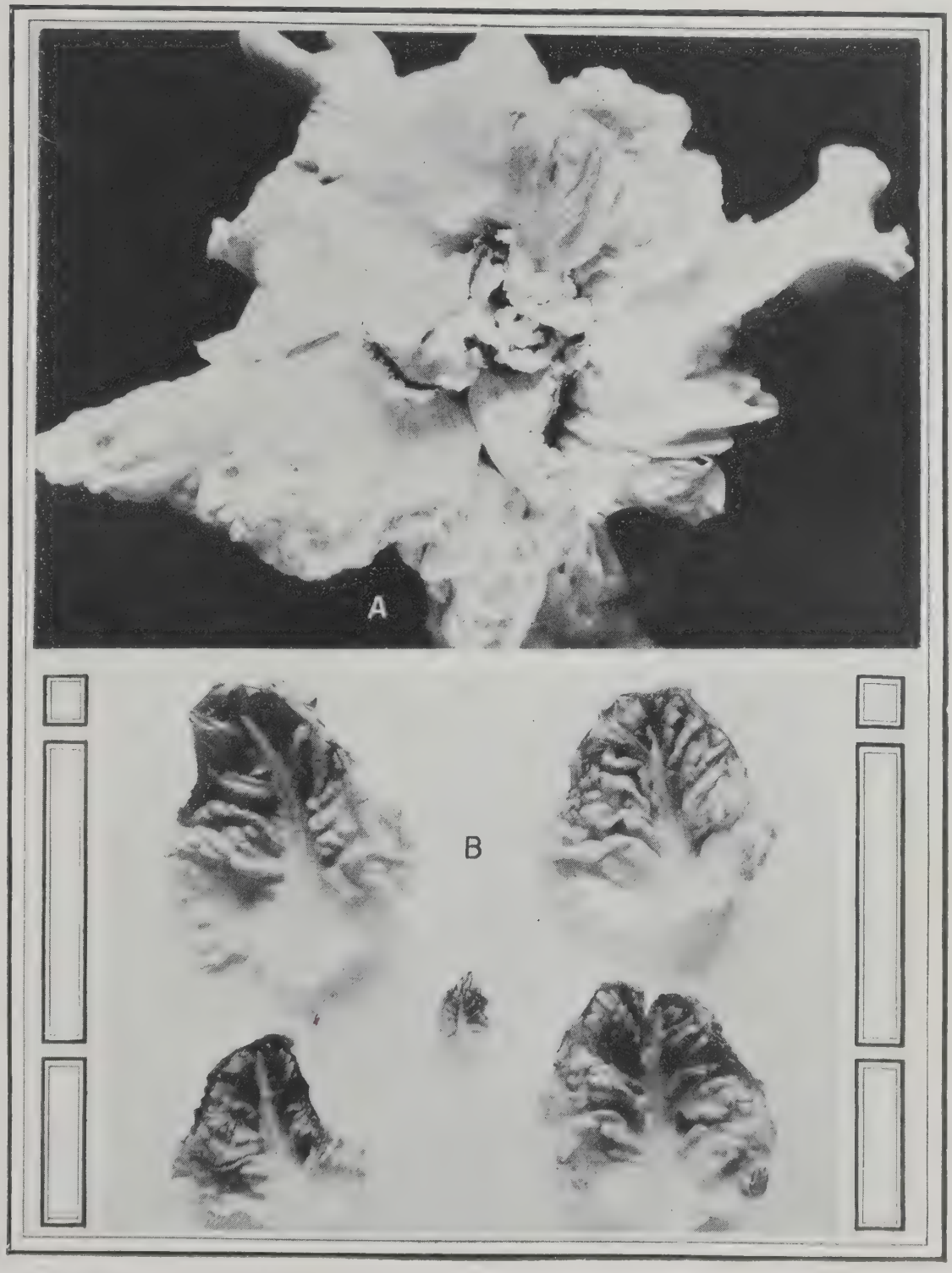

A
B

PLATE 34

Bacterium marginale:

A diseased leaf of lettuce received from Hutchinson, Kans. The organism producing this disease and that causing the marginal lettuce disease at Manhattan, Kans., are identical.

PLATE 35

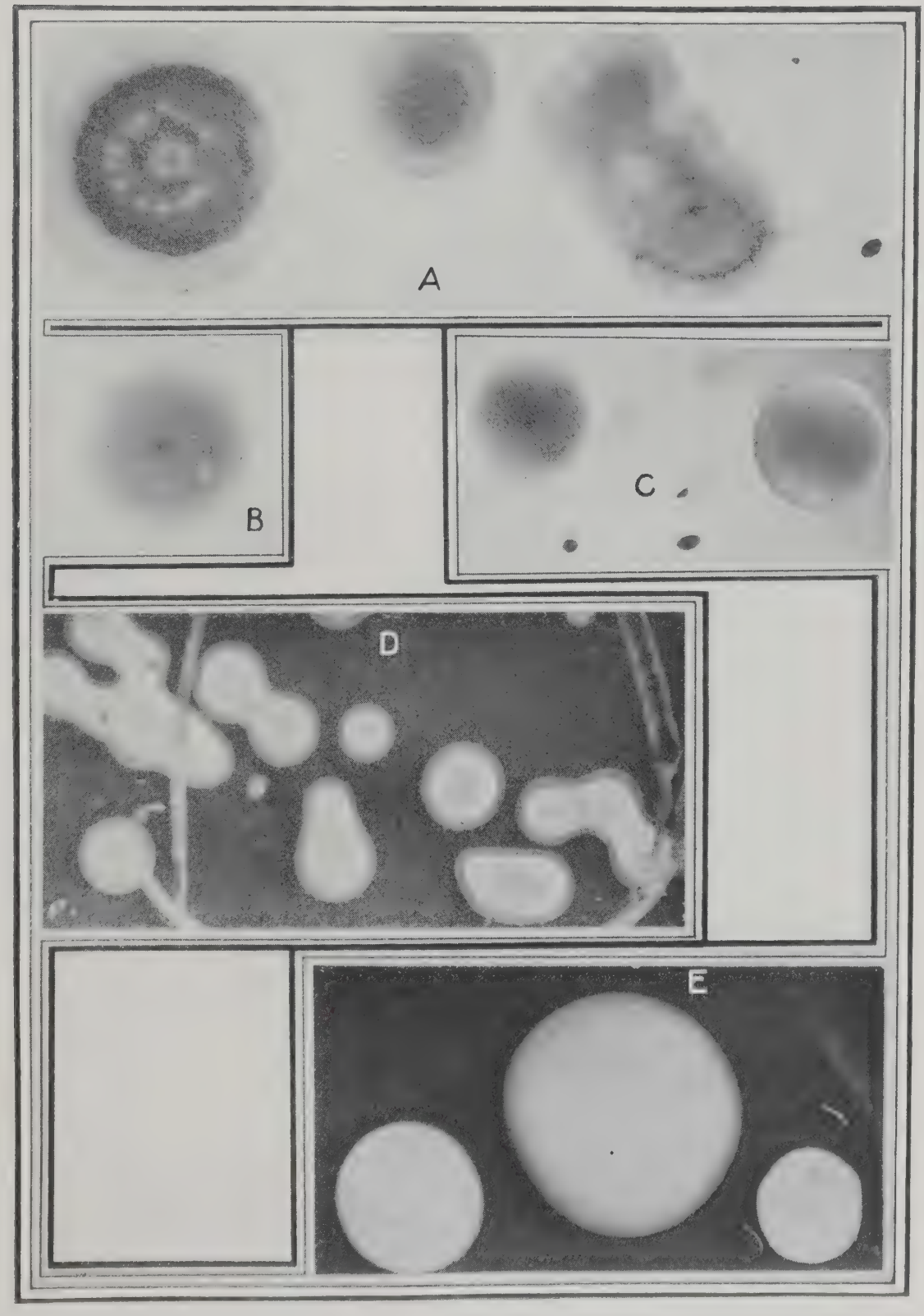
A.—Louisiana lettuce disease: Surface colonies on agar-poured plates of *Bacterium viridilividum*, showing the mottled type of colonies, and also one buried colony. Photographed at the end of six days. ×9.

B.—*Bact. viridilividum:* Nonmottled type three days after pouring. Both types of colonies are infectious. ×9.

C.—*Bact. vitians*, cause of the South Carolina lettuce disease: Agar-poured plate showing surface, buried, and bottom colonies. Photographed at end of the third day. ×9.

D.—*Bact. viridilividum*, cause of a Virginia lettuce disease: Mottled colonies on agar-poured plates. Photographed two days after pouring. The streak is a pencil mark on the outside of the dish. ×5.

E.—*Bact. marginale*, cause of the Kansas lettuce disease: Colonies on surface of agar-poured plates. Photographed three days after pouring. ×10.


A
B
C
D
E

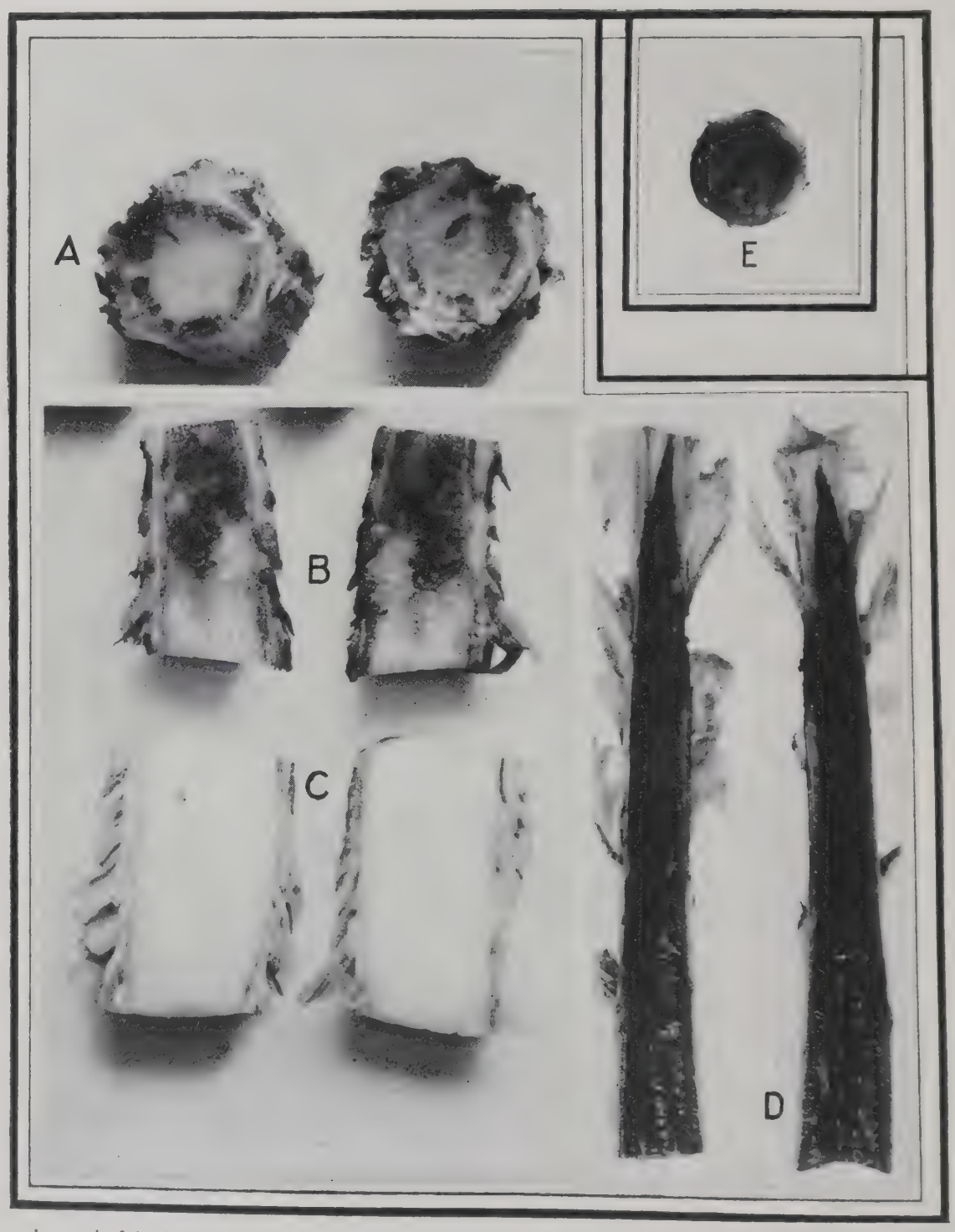

A
E
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PLATE 36

Bacterium vitians the cause of the South Carolina lettuce disease:

A.—Cross-sections of a lettuce stem at two levels 35 days after inoculation with the South Carolina yellow organism. The tissues are browned.

B.—A longitudinal section of another plant inoculated at the same time as A.

C.—A longitudinal section of a healthy stem for comparison.

D.—Longitudinal sections at the crown of a lettuce plant one month after inoculation, showing browning of the tissues.

E.—A cross section at the crown of a lettuce plant one month after inoculation, showing browning of the tissues.

PLATE 37

A.—Two leaves of a lettuce plant inoculated by spraying with *Bacterium viridilividum* isolated from Virginia lettuce. Photographed 37 days after inoculation.

B.—Cross sections of stems of lettuce plants inoculated with the Virginia yellow organism (*Bact. vitians*), which is the same as the South Carolina lettuce organism. At the right sections of two healthy stems are included.

A
B

A
B

PLATE 38

Bacterium vitians:

A.—A lettuce plant inoculated by spraying with the Virginia yellow organism, which is the same as the South Carolina yellow organism. Photographed one month after inoculation.

B.—Part of a healthy plant for comparison.

PLATE 39

Bacterium marginale:

A.—Part of a leaf from one of the original plants as received, showing the brown veins in the infected and shriveled margins. ×9 (about).

B.—Part of a lettuce leaf, showing the shriveling and the marginal brown venation produced by spraying with *Bact. marginale* on March 2, 1917. Photographed March 7, 1917. ×9 (about).

A
B

A
B

PLATE 40

Bacterium marginale:

A.—A head of lettuce showing the marginal infection on tender leaves in center. Inoculated by spraying on March 2, 1917. Photographed on March 16, 1917.

B.—Four lettuce leaves inoculated by spraying February 21, 1917. Photographed on February 23, 1917. Many of the infections are tiny spots not visible in the illustration—for example, on the leaf next to the smallest leaf at the right there are 75 such spots.

PLATE 41

A.—*Bacterium vitians:* Cross-section of stem showing bacteria in place. The organism has been stained with carbol fuchsin. ×1,000.

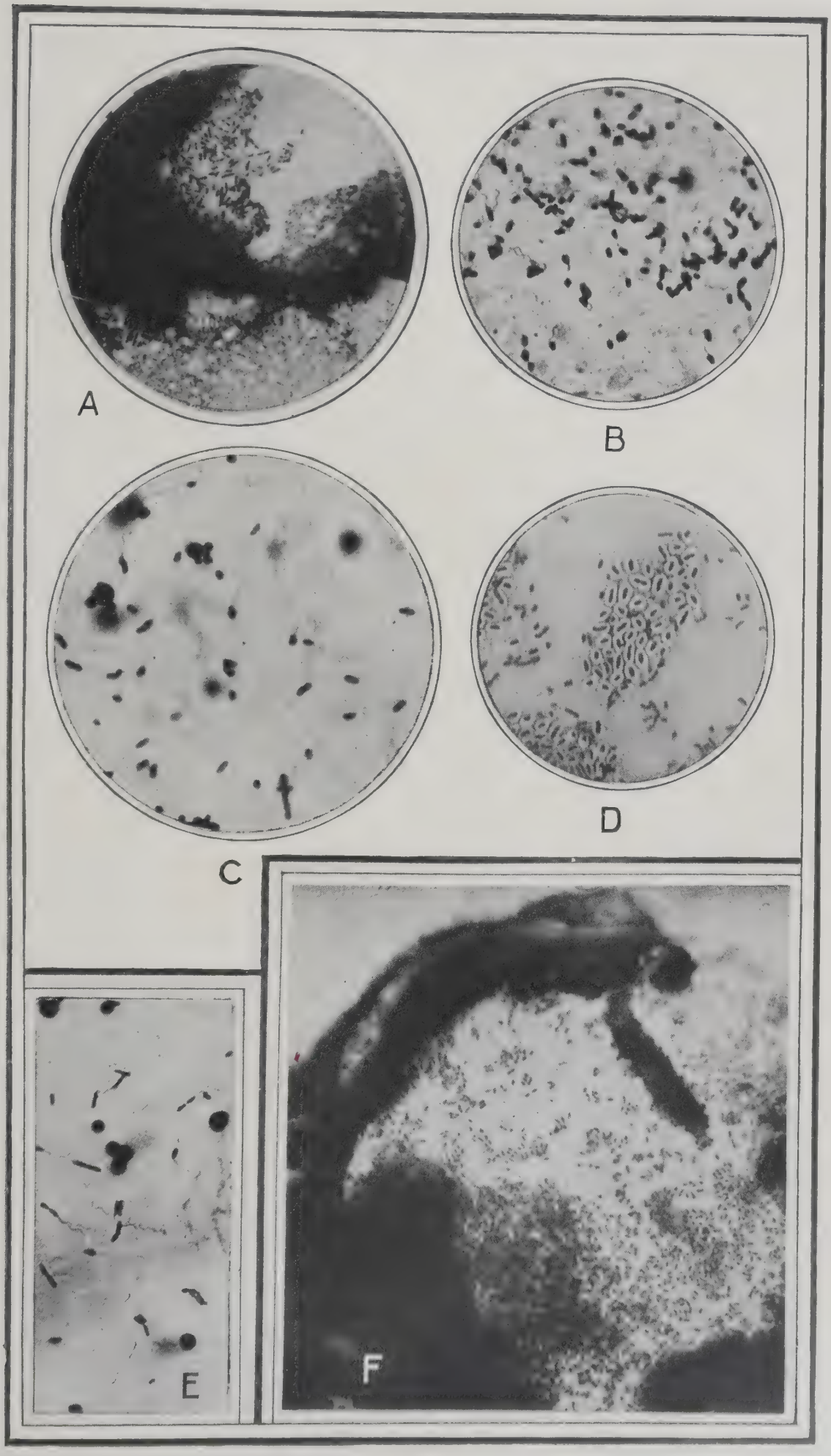
B.—*Bact. vitians:* Polar flagella stained with Casares-Gil's flagella stain; from a young agar culture. ×800 (about).

C.—*Bact. vitians* (Virginia): Polar flagella stained with Casares-Gil's flagella stain. Eighteen rods in this field bear flagella. ×800 (about).

D.—*Bact. marginale:* Grown on agar for two days and then stained with Ribbert's capsule stain. Photomicrographed by Dr. Erwin F. Smith. ×800 (about).

E.—*Bact. marginale:* Flagella stained with Casares-Gil's flagella stain. ×800 (about).

F.—*Bact. marginale:* Cross-section of a diseased, shriveled leaf showing bacteria in the tissues. Stained with carbol fuchsin. Photomicrographed by Dr. Erwin F. Smith. ×800 (about).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HYDRATION CAPACITY OF GLUTEN FROM "STRONG" AND "WEAK" FLOURS [1]

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INTRODUCTION

It is a well-known economic fact that there is a great variation in the baking quality of flours prepared from different wheats (*Triticum* spp.). The hard spring wheats, especially those of the northern portion of the Great Plains area, produce a flour which has superior baking qualities, while the softer wheats produce flour of inferior baking qualities. In order to differentiate between these qualities of the flour, the terms "strong" and "weak" flour have been generally accepted. For the purpose of this paper the definition (*8*)[3] adopted by a committee of the National Association of British and Irish Millers will be accepted—

> A strong wheat is one which yields flour capable of making large, well-piled loaves; the latter qualification thus excludes those wheats producing large loaves which do not rise satisfactorily.

Jago (*9, p. 291*) similarly defines "strength" as—

> the measure of the capacity of the flour for producing a bold, large-volumed, well-risen loaf.

Obviously those flours not meeting the above conditions must be classed as "weak."

HISTORICAL REVIEW

It is far beyond the scope of the present paper to enter into a complete historical discussion of the work which has been undertaken in attempts to ascertain what factors are responsible for the strength of flour. An enormous amount of literature has accumulated within the last 20 or 30 years, and this literature has been reviewed from time to time by workers

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[3] Reference is made by number (italic) to "Literature cited," p. 417–418.

in this field.[1] Almost every conceivable factor has been investigated with more or less thoroughness; yet—

> no one is believed to have discovered a limiting factor or group of factors which completely solves the problem (*3*).

Instead we wish to limit the discussion to those papers bearing directly upon our problem, the colloidal properties of the gluten.

It is only within comparatively recent years that the fact has been recognized that the physical state of matter is of paramount importance. When the nature of colloids was first investigated it was supposed that relatively few substances could form colloidal solutions or gels, but it now seems probable that under suitable conditions any substance may be obtained in colloidal form, and it appears almost equally probable that at some future date we may be able to obtain in crystalloidal form those substances which we now know only as colloids. When a substance passes from the crystalloidal to the colloidal state, the physical properties are so altered as to bear almost no resemblance to the original substance; and even when in the colloidal state, the properties are not constant but vary widely, depending upon the size of the colloidal particles, upon their electrical charges, and upon the presence or absence of foreign materials in the dispersion medium. This being the case, it does not necessarily follow that, when two colloidal preparations of the same material from different sources show identical chemical composition, they should also show identical physical behavior, for it is altogether possible that one preparation lies much nearer the boundary between the crystalloidal and colloidal states of matter than does the other.

Wood (*16*) and Wood and Hardy (*18*) have shown that wheat gluten is an emulsoid colloid. All proteins which have been investigated belong to this class. One of the most characteristic reactions of the emulsoids is that they have a great affinity for water, being sometimes classified as "hydrophylic" colloids. The degree of this affinity for water may be altered by the addition of salts, acids, or alkalis to the dispersion medium.

Hofmeister (*7*) was among the first to study the conditions causing colloidal swelling of proteins and other workers have extended his observations. Fischer (*4*) has summarized the data of these workers and added extensive experiments of his own. It has been found that the addition of an acid or an alkali causes a hydrophylic colloid to imbibe more water, which, if the colloid is in the form of a gel, results in a swelling of the material, and that this swelling can be more or less completely inhibited by the addition of salts.

Wood appears to have been the first to attribute the differences between strong and weak flours to the physical properties of their proteins

[1] One of the latest of these résumés is that of Blish (*3*). In his paper the various researches are reviewed and criticised, and new data are added.

rather than to chemical difference in the flours. His first paper (*16*) is devoted to a study of possible chemical differences. He determined total nitrogen, total gliadin nitrogen, amid nitrogen in the gliadin, ratio of gliadin to total protein, total ash, total soluble ash, acid, and carbon-dioxid production in several different flours. He contends that the size of the loaf is regulated to a large extent by the fermentable sugars which are present, this being indicated by the carbon dioxid evolved, while—

> shapeliness, and probably gas retention, are dependent on the physical properties of gluten as modified by the presence of varying proportions of salts.

In later papers Wood (*17*) and Wood and Hardy (*18*, *19*) investigated certain of the physical properties of gluten, especially as to the effect of electrolytes in solution upon its physical state.

That the gluten which is washed from a strong flour is different in character from that obtained from a weak flour is a matter of general knowledge. Shutt (*13*, *p. 60*) visualizes the differences between such glutens as follows:

> In flours of high bread-making values the gluten is resilient, elastic, firm, and cohesive; in poor flours it may be flabby, nonresilient, soft, or sticky.

Wood suspended strings of gluten about the size of a pencil across V-shaped glass rods in beakers containing varying concentrations of different acids and then noted the concentration at which cohesion was so far reduced as to allow the gluten to fall off the rod and disperse in a cloudy solution. He apparently used gluten from only one flour in these experiments.

In this manner it was found that gluten suspended in distilled water retained its coherence almost indefinitely, but that in solutions of hydrochloric acid as dilute as *N/1,000* dispersion began almost immediately. This action increased with an increased concentration of acid up to about *N/30*, and then decreased again until at a concentration of approximately *N/12* the gluten became—

> permanently coherent and much harder and more elastic, and less sticky than in its original condition.

Similar experiments were conducted with sulphuric, phosphoric, oxalic, acetic, lactic, citric, and tartaric acids, both with and without the additions of certain salts. Unfortunately Wood does not give the necessary tabular data to permit exact comparisons; and in the curve shown, those for "acid alone" are omitted, and only certain of those for "acid + salt" are given. He finds, however, that the order in which the acids affected the coherence of the gluten was (1) hydrochloric, (2) sulphuric, (3) phosphoric, (4) oxalic. The three remaining acids behaved quite differently, for, while dilute solutions caused disintegration, this

became increasingly rapid with increasing concentrations of acid, and no practicable concentration could be found at which coherence reappeared.[1]

Wood likewise observed that the addition of salts to the acid solution counteracted in a large measure the effect of the acid. He therefore postulated (*17, p. 272*) that—

> the variations in coherence, elasticity, and water content, observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the composition of the glutens themselves.

Wood and Hardy (*17, 18*) and Hardy (*6*) support this view in later papers.

Upson and Calvin (*14*) were the next to study the colloidal swelling of gluten. They employed a more exact technic than did Wood,[2] using the method employed by Hofmeister (*7*) in his investigations on the swelling of animal proteins. In their experiments the gluten was first freed from starch by washing it in a stream of distilled water. It was then pressed out between glass plates to a fairly uniform thickness and, after standing for some time, was cut into small disks. These disks were weighed to the nearest centigram, placed in beakers containing acid solutions of varying concentrations, and allowed to remain for a constant period of time. They were then removed, drained, and reweighed. The increase in weight due to imbibition of water was calculated to the amount imbibed per gram of moist gluten. The experiments were then repeated, except that a series of salts was added to the different concentrations of the acids. The addition of the salts caused a diminution of the water imbibition. They found that in dilute acids the gluten swells and—

> the disks puff up and take on an appearance somewhat resembling cotton balls, finally becoming transparent, soft, and gelatinous.

They furthermore found that the taking up and giving off of water was largely reversible and, by neutralizing the acid after swelling of the disks had taken place, it would lose water and again become a firm coagulum.

In a later publication (*15*) the same investigators give results of further studies on the colloidal swelling of wheat gluten as related to baking strength of flour and conclude that—

> strength is related to soluble acid and salt content of the flour. Flours containing acids and salts in such combinations as to favor water absorption will behave as "weak" flours, whereas those containing acids and salts in such combinations as inhibit water absorption will behave as strong flours when baked.

---

[1] Fischer (*4*) gives a different order for the effectiveness of acids causing swelling of animal proteins—that is, (1) hydrochloric, (2) phosphoric, (3) lactic, (4) formic, (5) oxalic, (6) nitric, (7) acetic, (8) citric, (9) sulphuric. The noteworthy differences between these two lists lies in the relative position of sulphuric acid.

[2] Wood allowed the gluten to imbibe water until it lost coherence and began to disperse as a sol. Upson and Calvin, on the other hand, determined the weight of the water imbibed by the gluten in a fixed period of time and before imbibition had progressed far enough to cause dispersion.

It should be noted that this finding is in many respects similar to that of Wood (*17*):

> The variations in coherence, elasticity, and water content, observed in gluten extracted from different flours, are due rather to varying concentrations of acid and soluble salts in the natural surroundings of the gluten than to any intrinsic difference in the glutens themselves.

There are, however, marked differences between these two statements; these will be considered later in this paper.

EXPERIMENTAL WORK

THE PROBLEM

In the experiments reported by Wood and in those recorded by Upson and Calvin it would appear that in each instance the action of acids and salts had been tested upon gluten derived from only one flour, and this presumably a "strong" flour, and that the application of such data to the problem of flour strength was made by analogy rather than actual observation. It was thought worth while, therefore, to repeat the work of Upson and Calvin (*14*, *15*), with glutens derived from flours of widely differing baking strength in order to determine what correlation, if any, exists between the baking qualities of the flour and the hydration capacity of the gluten.

MATERIAL USED

Five different flours were used. The first, a typical Minneapolis patent grade milled from No. 1 northern hard spring wheat from the 1916 crop. The second was a first clear grade milled from the 1915 crop. The three other samples were milled in the State of Oregon from typical soft wheats grown in that section, and are "straight-grade" flours. It is to be regretted that sufficient amounts of two of the western flours, W_1 and W_2, were not available for all of the experimental work.

METHOD OF EXPERIMENTATION

The method used in studying these flours was the same as that used by Hofmeister in his work on the swelling of gelatin and, as mentioned above, by Upson and Calvin. Briefly, it consisted in first doughing 200 gm. of flour by adding the required amount of distilled water. The dough was then permitted to stand for from 30 minutes to an hour under distilled water, after which it was washed for 15 minutes under a stream of distilled water. Almost all of the starch was washed out in this manner. The gluten was then submerged under distilled water until all the desired samples of gluten had been prepared.

It was interesting to note the difference in the character of the gluten prepared from the different samples. The patent flour, from No. 1 northern grade of wheat, called "P," gave a rather firm coherent gluten, as did the first clear grade, "C." The three western flours, designated "W^1," "W^2," and "W^3," gave a more friable, sticky, and less coherent gluten which was much more difficult to obtain than was the gluten from

the two other flours, so greatly was it lacking in coherency. The gluten from each of these flours was then pressed out to a nearly uniform thickness of about 3 mm. between glass plates, and after draining for a few minutes (this interval of time was kept as nearly constant as possible), was cut into small disks by means of a large cork borer. These disks, which were fairly uniform as to size, shape, and weight, were weighed out to an accuracy of 5 mgm., placed in acid solutions of varying compositions and concentrations, and left for exactly 50 minutes. They were then removed, drained for about 10 minutes on a perforated porcelain plate and reweighed. The change in weight was calculated to the change per gram of moist gluten. Preliminary experiments were undertaken in order to determine the maximum time during which glutens

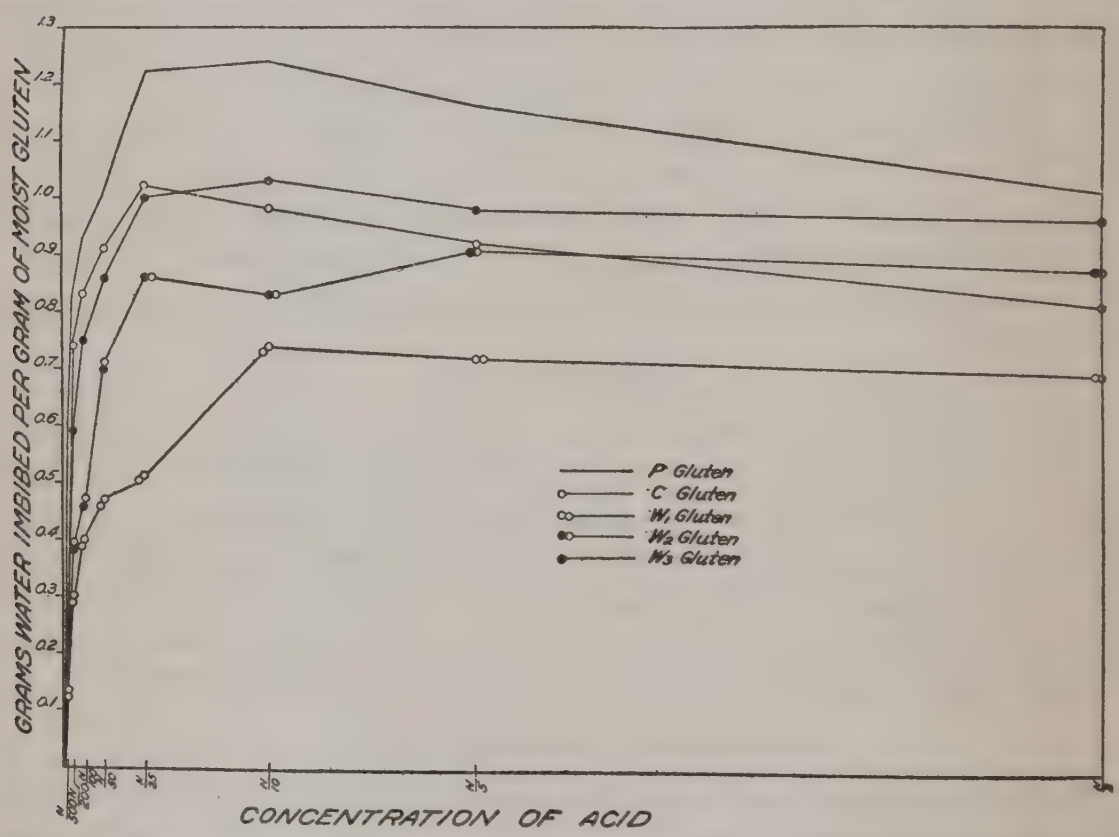

FIG. 1.—Graph showing the imbibition curves for the various glutens in different concentrations of lactic acid.

from all the flours could remain submerged in the different concentrations of the various acids and still retain their coherence sufficiently to make weighing possible. This time was found to be 50 minutes. In order that the results with the different flours might be comparable, this time interval was kept exactly the same in all the experiments reported in the following tables. The glutens for any given set of experiments were prepared at the same time and placed in acids at the same temperature. Every possible precaution was taken to eliminate variations in experimental conditions, and we believe that any appreciable difference between the recorded constants may be attributed to intrinsic differences in the various glutens themselves.

This method of measuring water imbibition or "hydration capacity" is obviously somewhat crude when compared with the usual chemical

procedure, but the errors are reduced to a minimum by taking the average of several different determinations.

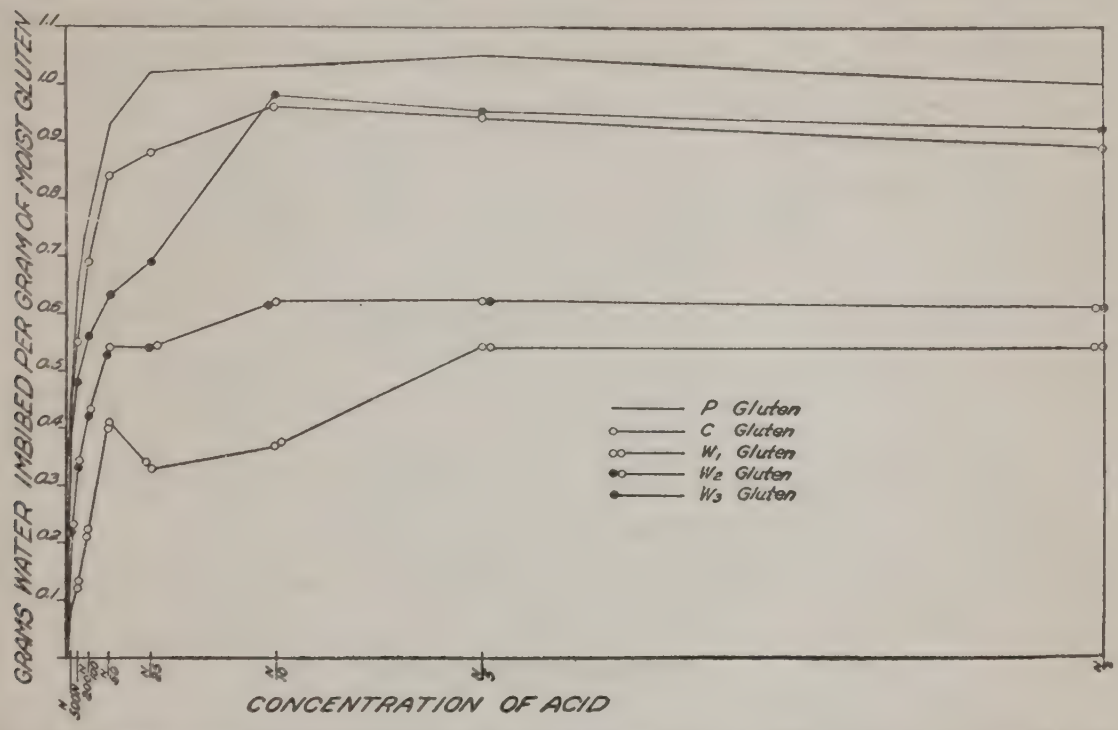

FIG. 2.—Graph showing the imbibition curves for the various glutens in different concentrations of acetic acid.

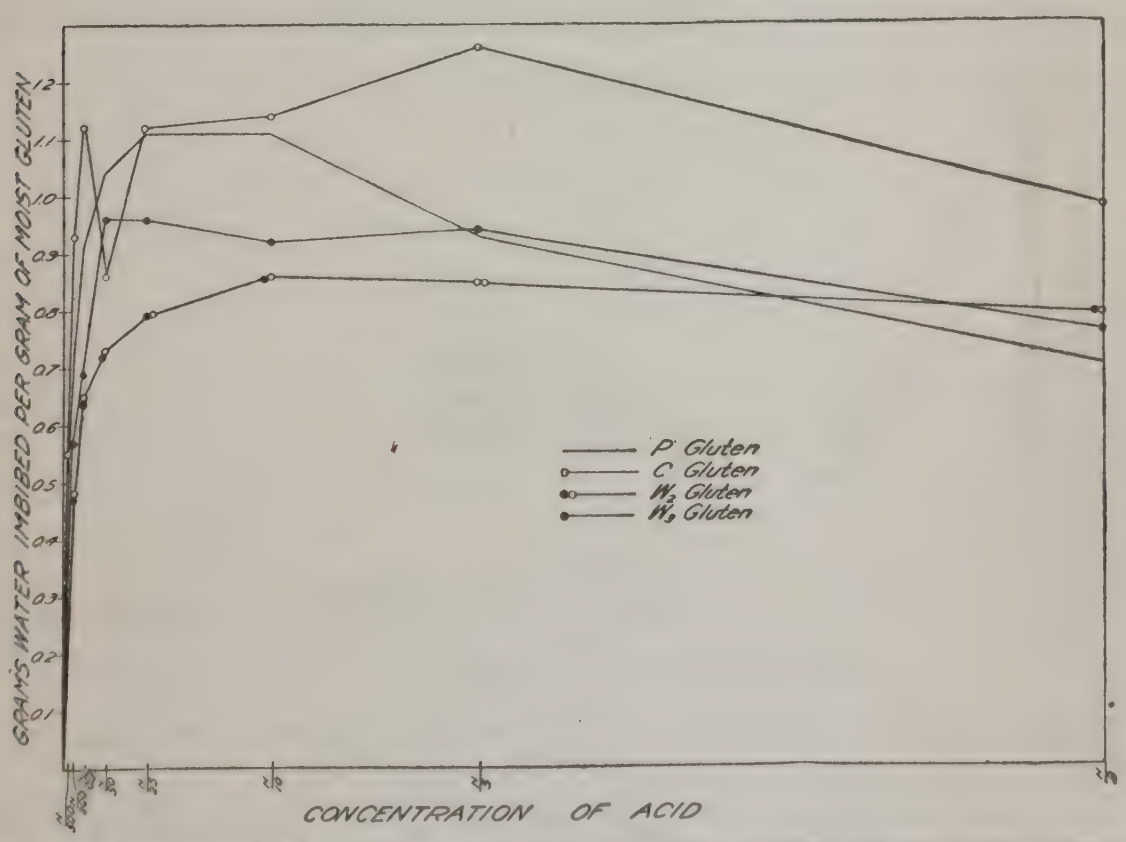

FIG. 3.—Graph showing the imbibition curves for the various glutens in different concentrations of orthophosphoric acid.

It will be observed that the figures in Tables I to V are all average values of from three to seven individual determinations. It was not thought worth while to record all of the individual values. The agree-

ment between duplicates was, however, of the same order as that shown in Upson and Calvin's tables (*14*, *15*).

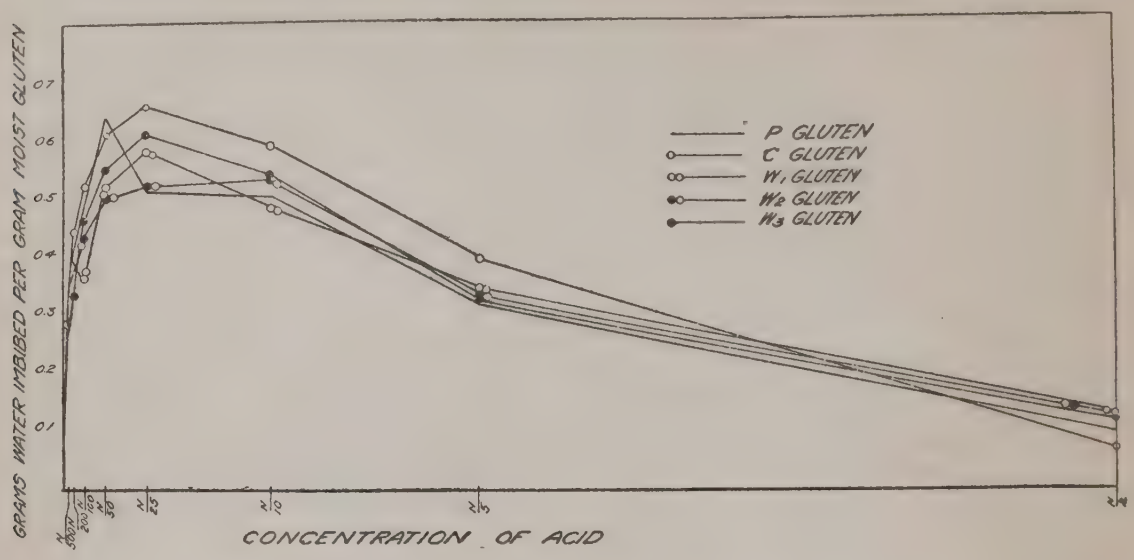

FIG. 4.—Graph showing the imbibition curves for the various glutens in different concentrations of oxalic acid.

EXPERIMENTAL DATA

(a) IMBIBITION OF WATER BY THE DIFFERENT GLUTENS IN THE PRESENCE OF DILUTE ACIDS.—The amount of water imbibed by the different glutens under the above conditions has been measured in eight

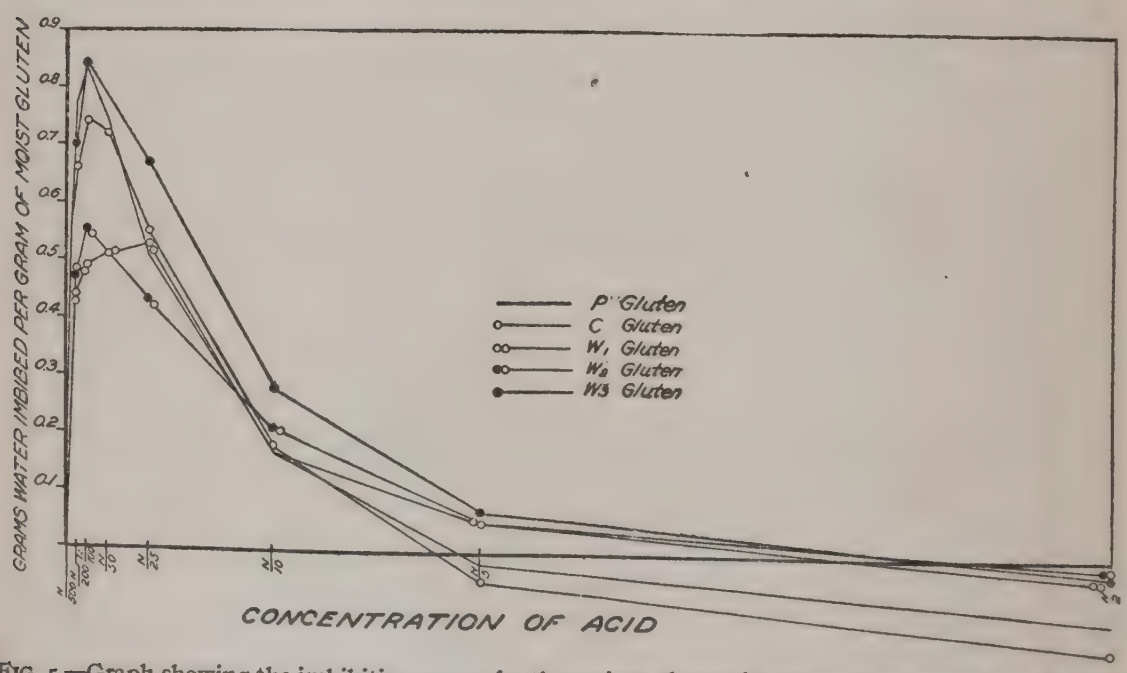

FIG. 5.—Graph showing the imbibition curves for the various glutens in different concentrations of hydrochloric acid.

concentrations of each of the following acids: hydrochloric, oxalic, orthophosphoric, lactic, acetic, and boric. The average data obtained from these experiments are shown in the first five columns of Tables I to IV and in Table V. The results are shown graphically in figures 1 to 5. The values for boric acid have not been plotted, inasmuch as it appears that boric acid has little or no effect on water imbibition by gluten.

TABLE I.—*Relative imbibition of the glutens from patent, clear, and western flours in lactic acid and in lactic acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).																										
	Lactic acid alone.				Lactic acid + $\frac{M}{200}$ potassium chlorid.				Lactic acid + $\frac{M}{200}$ potassium phosphate.			Lactic acid + $\frac{M}{200}$ potassium tartrate.			Lactic acid + $\frac{M}{200}$ calcium chlorid.			Lactic acid + $\frac{M}{200}$ mercuric chlorid.			Lactic acid + $\frac{M}{200}$ aluminium sulphate.			Lactic acid + $\frac{M}{200}$ magnesium sulphate.		
	W_1.	W_2.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.
None.....	0.00	−0.01	−0.01	0.01	−0.01	−0.02	−0.24	−0.04	−0.08	−0.13	−0.05	0.04	0.05	0.07	−0.08	−0.16	−0.03	0.56	0.50	0.31	0.06	0.01	0.09	−0.08	−0.20	−0.04
N/500.....	.12	.23	.63	.65	.45	.22	.14	.18	.22	.22	.21	.10	.10	.15	.02	−.07	.09	.44	.49	.21	.09	.05	.12	−.06	−.18	.00
N/200.....	.29	.38	.82	.74	.59	.38	.39	.33	.38	.31	.32	.31	.35	.24	.25	.30	.25	.42	.47	.19	.15	.14	.14	.03	−.07	.07
N/100.....	.40	.46	.93	.83	.75	.55	.55	.39	.50	.67	.54	.41	.54	.36	.42	.43	.38	.37	.47	.18	.22	.26	.17	.12	.09	.13
N/50.....	.47	.71	1.10	.91	.86	.66	.65	.54	.63	.76	.62	.73	.74	.48	.48	.56	.43	.37	.30	.15	.28	.33	.24	.23	.29	.23
N/25.....	.51	.86	1.22	1.02	1.00	.79	.73	.61	.77	.79	.78	.77	.81	.65	.60	.57	.54	.33	.25	.13	.36	.38	.31	.35	.44	.30
N/10.....	.74	.83	1.24	.98	1.03	.94	.90	.75	.83	.93	.88	.92	.85	.74	.73	.72	.64	.30	.27	.14	.46	.39	.35	.53	.52	.41
N/5.....	.71	.91	1.16	.92	.98	1.05	.85	.85	.90	.97	.83	.95	1.11	.81	.83	.83	.73	.32	.20	.16	.54	.55	.43	.66	.59	.48
N/2.....	.69	.87	1.01	.81	.96	.96	.78	.83	.93	.93	.81	1.01	.91	.76	.83	.83	.94	.33	.19	.10	.57	.58	.44	.64	.49	.56

[1] The figures for P and C flours in the acid alone controls are the averages of six different determinations; those for W_1 and W_2 are the averages of three; and those for W_3 are the averages of seven different determinations. The figures for acid plus the various salts are in every case averages of three different determinations.

TABLE II.—*Relative imbibition of the glutens from patent, clear, and western flours in hydrochloric acid and in hydrochloric acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).																									
	Hydrochloric acid alone.					Hydrochloric acid + $\frac{M}{200}$ potassium chlorid.			Hydrochloric acid + $\frac{M}{200}$ potassium phosphate.			Hydrochloric acid + $\frac{M}{200}$ potassium tartrate.			Hydrochloric acid + $\frac{M}{200}$ calcium chlorid.			Hydrochloric acid + $\frac{M}{200}$ mercuric chlorid.			Hydrochloric acid + $\frac{M}{200}$ aluminium sulphate.			Hydrochloric acid + $\frac{M}{200}$ magnesium sulphate.		
	W_1.	W_2.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.	P.	C.	W_3.
None	−0.03	0.01	−0.02	−0.06	0.00	−0.09	−0.13	−0.03	−0.09	−0.07	−0.03	0.04	0.07	0.09	−0.05	−0.18	−0.01	0.66	0.50	0.26	0.02	0.02	0.06	−0.05	−0.24	−0.04
N/500	.27	.34	.57	.59	.52	.31	.20	.24	.35	.33	.33	.16	.18	.21	.24	.16	.26	.53	.34	.35	.10	.10	.10	.01	−.13	.04
N/200	.44	.47	.77	.66	.70	.42	.39	.43	.58	.49	.49	.30	.34	.35	.41	.38	.45	.58	.56	.36	.19	.22	.17	.13	.08	.17
N/100	.49	.55	.83	.74	.84	.67	.51	.55	.66	.61	.62	.58	.56	.53	.51	.59	.54	.69	.61	.55	.29	.35	.24	.27	.23	.22
N/50	.51	.51	.74	.72	.81	.68	.55	.67	.67	.63	.63	.59	.58	.61	.46	.40	.45	.62	.65	.60	.38	.36	.35	.34	.33	.35
N/25	.53	.43	.51	.55	.67	.49	.42	.50	.57	.50	.56	.47	.51	.52	.48	.51	.47	.38	.32	.45	.34	.39	.31	.32	.28	.32
N/10	.17	.21	.17	.18	.28	.17	.12	.21	.19	.14	.22	.16	.19	.24	.15	.06	.20	.08	.03	.12	.15	.15	.16	.18	.04	.18
N/5	.05	.05	−.02	−.05	.07	−.01	−.08	.04	−.01	−.09	.05	.02	.04	.04	.00	−.23	.04	−.02	−.07	.02	.03	−.05	.07	.03	−.03	.04
N/2	−.04	−.02	−.11	−.16	−.03	−.13	−.22	−.04	−.18	−.25	−.07	−.05	−.08	−.03	−.10	−.34	−.03	−.07	−.18	−.03	−.07	−.20	−.03	−.06	−.30	−.03

[1] These figures are the averages of the same number of determinations in each case as with the corresponding columns in Table I.

TABLE III.—*Relative imbibition of the glutens from patent, clear, and western flours in acetic acid and in acetic acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).													
	Acetic acid alone.					Acetic acid + $\frac{M}{200}$ potassium chlorid.			Acetic acid + $\frac{M}{200}$ potassium phosphate.			Acetic acid + $\frac{M}{200}$ potassium tartrate.		
	W_1	W_2	P	C	W_3	P	C	W_3	P	C	W_3	P	C	W_3
None....	0.01	0.00	0.01	0.01	0.03	−0.10	−0.14	−0.06	−0.04	−0.04	−0.04	0.02	−0.08	0.09
N/500...	.08	.22	.48	.45	.39	.07	.06	.11	.18	.21	.18	.05	.03	.12
N/200...	.12	.33	.65	.65	.48	.20	.21	.24	.28	.29	.32	.13	.12	.17
N/100...	.21	.42	.76	.69	.56	.30	.42	.30	.39	.37	.29	.24	.31	.24
N/50....	.41	.54	.93	.84	.63	.42	.67	.37	.41	.48	.38	.30	.51	.30
N/25....	.33	.54	1.02	.88	.69	.51	.71	.49	.54	.56	.45	.49	.56	.36
N/10....	.37	.62	1.03	.96	.98	.58	.76	.55	.66	.64	.55	.65	.62	.46
N/5.....	.54	.62	1.05	.94	.95	.62	.75	.64	.77	.68	.60	.62	.75	.53
N/2.....	.54	.61	1.00	.89	.92	.72	.72	.64	.77	.69	.65	.74	.80	.60

[1] The figures for P and C flours in the acid alone controls are the averages of six different determinations. All of the remaining in both acid alone, and acid plus salts are averages of three different determinations.

TABLE IV.—*Relative imbibition of the glutens from patent, clear, and western flours in oxalic acid and in oxalic acid plus certain salts.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).													
	Oxalic acid alone.					Oxalic acid + $\frac{M}{200}$ potassium chlorid.			Oxalic acid + $\frac{M}{200}$ potassium phosphate.			Oxalic acid + $\frac{M}{200}$ potassium tartrate.		
	W_1	W_2	P	C	W_3	P	C	W_3	P	C	W_3	P	C	W_3
None.....	−0.01	−0.01	−0.02	−0.03	0.02	−0.11	−0.11	−0.04	−0.04	−0.10	−0.08	0.03	−0.02	0.08
N/500.....	.23	.27	.33	.29	.26	.12	.17	.16	.21	.16	.20	.08	.04	.11
N/200.....	.40	.38	.42	.45	.34	.22	.24	.24	.38	.32	.31	.14	.14	.18
N/100.....	.37	.44	.50	.53	.47	.36	.38	.38	.51	.40	.42	.31	.27	.24
N/50.....	.53	.51	.65	.62	.56	.47	.50	.47	.64	.54	.55	.44	.49	.40
N/25.....	.59	.53	.52	.67	.62	.53	.63	.48	.70	.70	.64	.53	.71	.51
N/10.....	.49	.54	.51	.60	.55	.41	.49	.44	.61	.63	.58	.53	.61	.49
N/5.....	.35	.34	.32	.40	.33	.26	.22	.24	.37	.35	.35	.32	.48	.32
N/2.....	.13	.13	.10	.07	.12	.06	.05	.11	.12	.09	.13	.11	.17	.15

[1] These figures are the average of the same number of determinations in each case as with the corresponding columns in Table III.

TABLE V.—*Relative imbibition of the glutens from patent, clear, and western flours in ortho-phosphoric and boric acids.*[1] *Temperature 24° C.*

Concentration of acid.	Quantity of water absorbed (grams per gram of moist gluten).							
	Ortho-phosphoric acid.				Boric acid.			
	P	C	W_2	W_3	P	C	W_2	W_3
None.....	−0.02	0.03	−0.04	−0.01	−0.02	−0.10	−0.01	−0.02
N/500.....	.53	.55	.18	.40	−.04	−.10	.00	−.02
N/200.....	.71	.93	.47	.57	−.05	−.06	−.01	−.01
N/100.....	.92	1.12	.65	.69	−.05	−.05	−.02	.01
N/50.....	1.04	.86	.73	.96	−.05	−.06	−.02	−.01
N/25.....	1.11	1.12	.79	.96	−.05	−.07	−.02	−.02
N/10.....	1.11	1.14	.86	.92	−.04	−.06	−.01	−.02
N/5.....	.93	1.26	.85	.94	−.04	−.08	−.01	.00
N/2.....	.70	.98	.79	.76	−.05	−.09	−.01	−.04

[1] These figures are the averages of three different determinations.

ANTAGONISTIC ACTION OF SALTS UPON THE IMBIBITION OF WATER BY THE VARIOUS GLUTENS IN THE PRESENCE OF HYDROCHLORIC, OXALIC, LACTIC, AND ACETIC ACIDS.—The amount of water imbibed by the P, C, and W_3 glutens was again measured as under (a), with the exception that various salts in 0.005 molar concentration were added to the acid solutions. The salts used were potassium chlorid (KCl), potassium phosphate (KH_2PO_4), potassium tartrate ($KHC_4H_4O_6$), calcium chlorid ($CaCl_2 + 2H_2O$), mercuric chlorid ($HgCl_2$), magnesium sulphate ($MgSO_4 + 7H_2O$), and aluminium sulphate $Al_2(SO_4)_3$. All of these salts were used with hydrochloric and lactic acids, but only the first three with oxalic and acetic

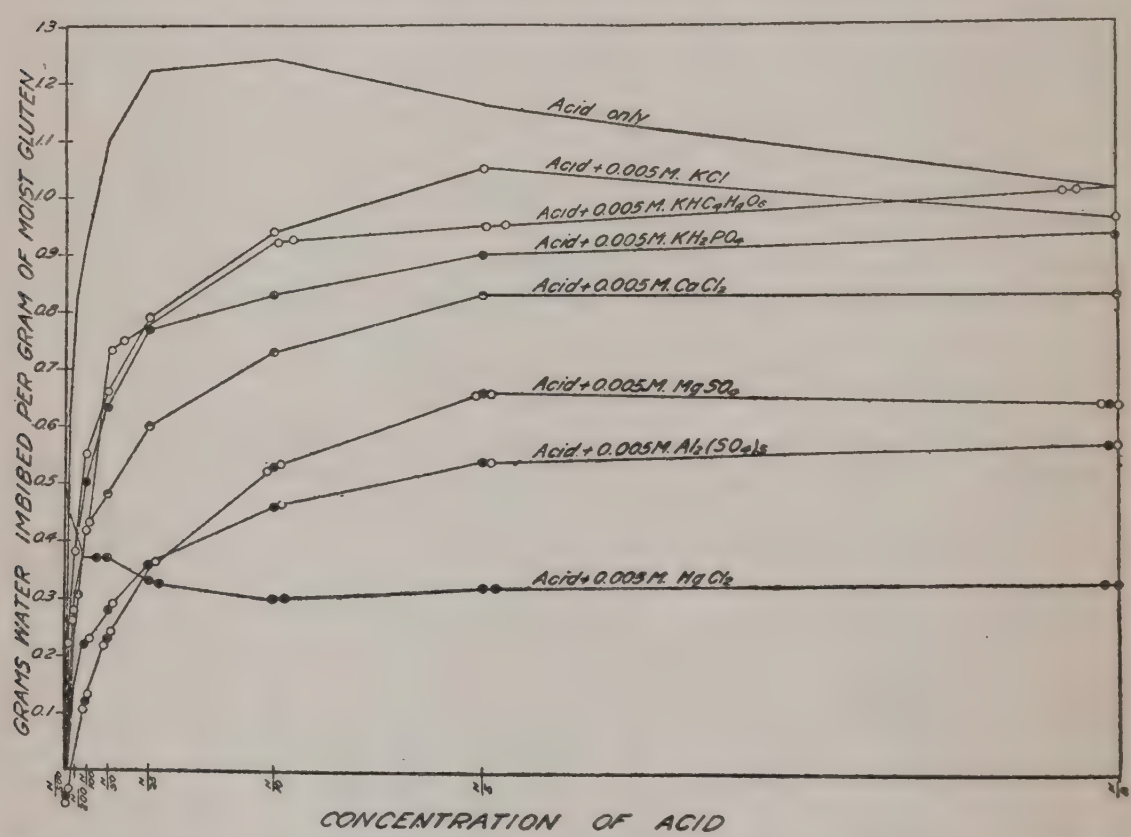

FIG. 6.—Graph showing the imbibition curves for P gluten in lactic acid and in lactic acid plus certain salts.

acids. The quantity of water, in grams, imbibed per gram of moist gluten under these conditions is given in the latter columns of Tables I to IV. It is physically impossible to visualize the data given in these tables without the aid of graphs. The corresponding curves were accordingly constructed and are shown in figures 6 to 17.

(c) FLOUR ANALYSES AND BAKING TESTS.—In Table VI are shown the following data determined from either the analysis of the flour or from baking tests: Ash on dry flour as determined by ignition in platinum in a muffle furnace, ash in an aqueous extract of the flour,[1] ratio of "soluble ash" to

[1] 100 c.c. of carbon-dioxid-free water containing a few drops of toluene were added to 25 gm. of flour; the mixture was thoroughly stirred and allowed to stand for three hours, after which the solution was filtered off for the "soluble-ash" and specific-conductivity determinations.

"total ash" in the flours, percentage of wet gluten and of dry gluten in the flours, percentage of ash in the dry gluten, specific conductivity of the flour extract at 25° C., water added to make the dough, volume of loaf, texture of loaf, and expansimeter test (*2*).

TABLE VI.—*Relative flour analyses and baking tests on P, C, W_1, W_2, and W_3 flours*

Mark.	Ash.		Soluble ash.	Crude gluten.		Ash in dry gluten.	Specific conductivity.	Water used per 100 gm. of flour.	Volume of loaf.	Texture.	Expansimeter test.
	Dry matter.	Water extract.	Total ash.	Wet.	Dry.						
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*		*C. c.*	*C. c.*		*C. c.*
P	0.46	0.27	59.32	30.23	10.02	0.31	1.08×10^{-3}	60.0	1,440	100	870
C	.56	.33	58.78	32.39	10.57	.32	1.22×10^{-3}	58.2	1,405	95	775
W_1	.61	.40	64.22	24.11	7.68	.55	1.46×10^{-3}	56.0	1,345	97	650
W_2	.62	.37	55.40	19.56	6.66	.42	1.29×10^{-3}	57.1	1,220	96	620
W_3	.49	.30	61.00	25.45	7.28	.39	1.04×10^{-3}	57.5	1,320	95	620

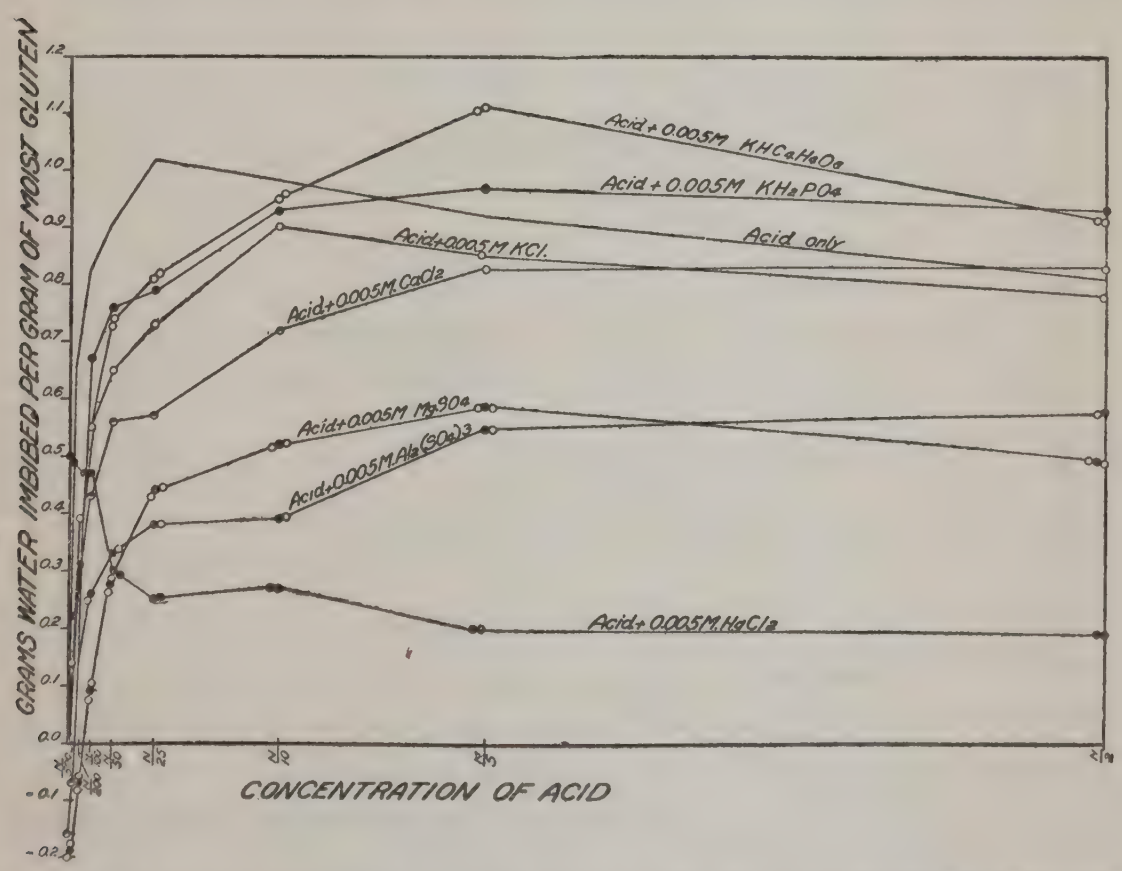

FIG. 7.—Graph showing the imbibition curves for C gluten in lactic acid and in lactic acid plus certain salts.

The notes taken during the progress of the baking test are as follows:

W_3 rose much more slowly in the expansimeter. In No. W_1, W_2, and W_3 the loaf was to some extent too compact and solid, apparently because of the fact that the gluten was not sufficiently tenacious to retain the carbon dioxid against the weight of the loaf above it. This was especially marked in W_2, where the lower third of the loaf was almost entirely lacking in porosity and light, uniform, velvety appearance.

RELATION BETWEEN THE QUALITY OF THE VARIOUS GLUTENS AND THEIR DEGREE OF HYDRATION

A study of the preceding tables and graphs confirms certain of the findings of Upson and Calvin, that—

gluten is an emulsoid colloid and shows all the properties of this class of compounds

and that—

gluten absorbs water from dilute acid solutions, thereby losing its tenacity and ductility, becoming soft and gelatinous. The presence of small amounts of neutral salts in the dilute acid solutions inhibits water absorption by gluten.

The data, however, do not support the latter part of their third statement that—

the bread-making qualities of dough made from wheat flours are dependent on the quantity and quality of the contained gluten. Quality of gluten is regulated by the

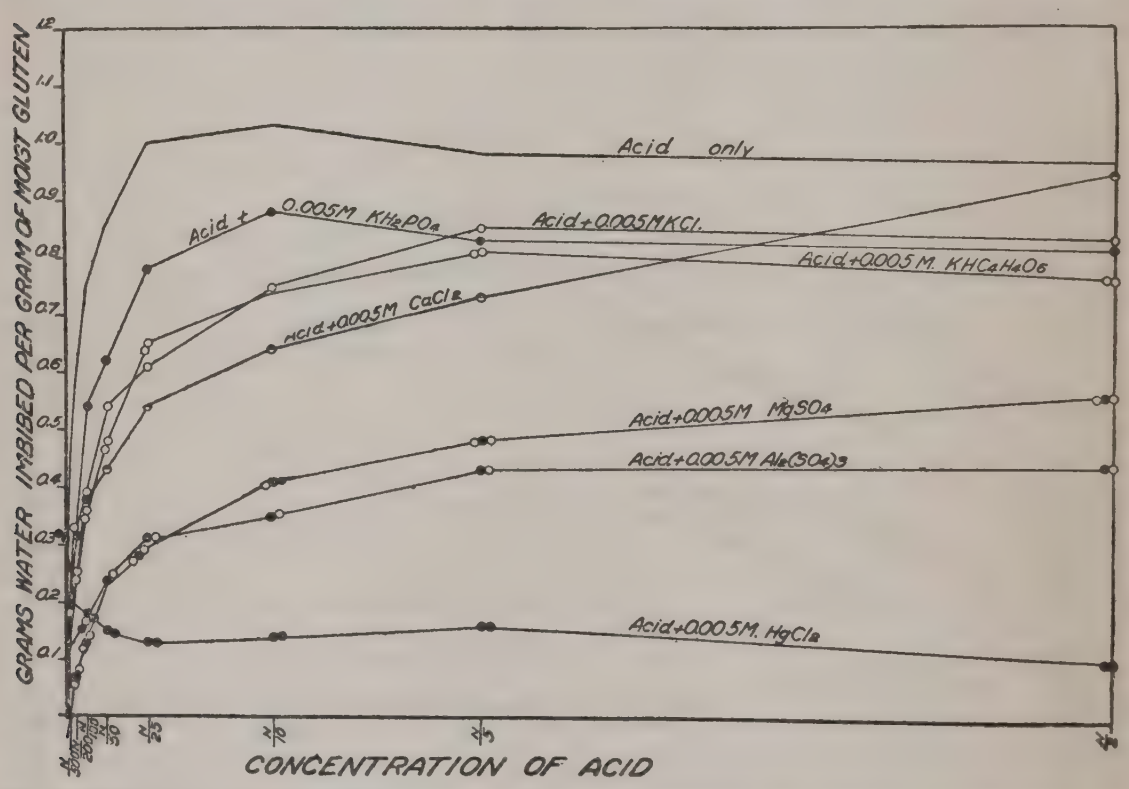

FIG. 8.—Graph showing the imbibition curves for W3 gluten in lactic acid and in lactic acid plus certain salts.

kind and concentration of the acids and salts present in the dough. If the kinds and amounts of the acids and salts are such as to favor water absorption, the quality of the gluten will be poor, whereas the presence of acids and salts in such amounts as tend to inhibit water absorption makes for an improved gluten,

but, on the contrary, all the evidence is directly opposed to such a conclusion.

The above statements by Upson and Calvin seem to have only one interpretation: that a weak gluten is weak because it is hydrated to a greater extent than is a strong gluten, and that in this respect, and in this respect only, does a weak gluten differ from a strong gluten. Upson and Calvin present data in their own bulletin which would have refuted this idea had they made the necessary calculations.

In Table VI are presented the percentages of moist gluten and dry gluten in the five flours which we have studied. If now we use these figures to calculate the percentage of water in the moist gluten, or, in other words, the amount of water imbibed by the dry gluten in preparing the gluten for the tests, we find the figures given in Table VII.

TABLE VII.—*Percentage of water in the moist gluten of various flours*

Mark.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if glutens were hydrated equal to P.	Difference of actual from hypothetical water content.
P	66. 85	33. 15	.....	.....
C	67. 37	32. 63	65. 82	+ 1. 55
W_1	68. 15	31. 85	64. 24	+ 3. 89
W_2	66. 00	34. 00	68. 56	− 2. 56
W_3	71. 39	28. 61	57. 69	+13. 70

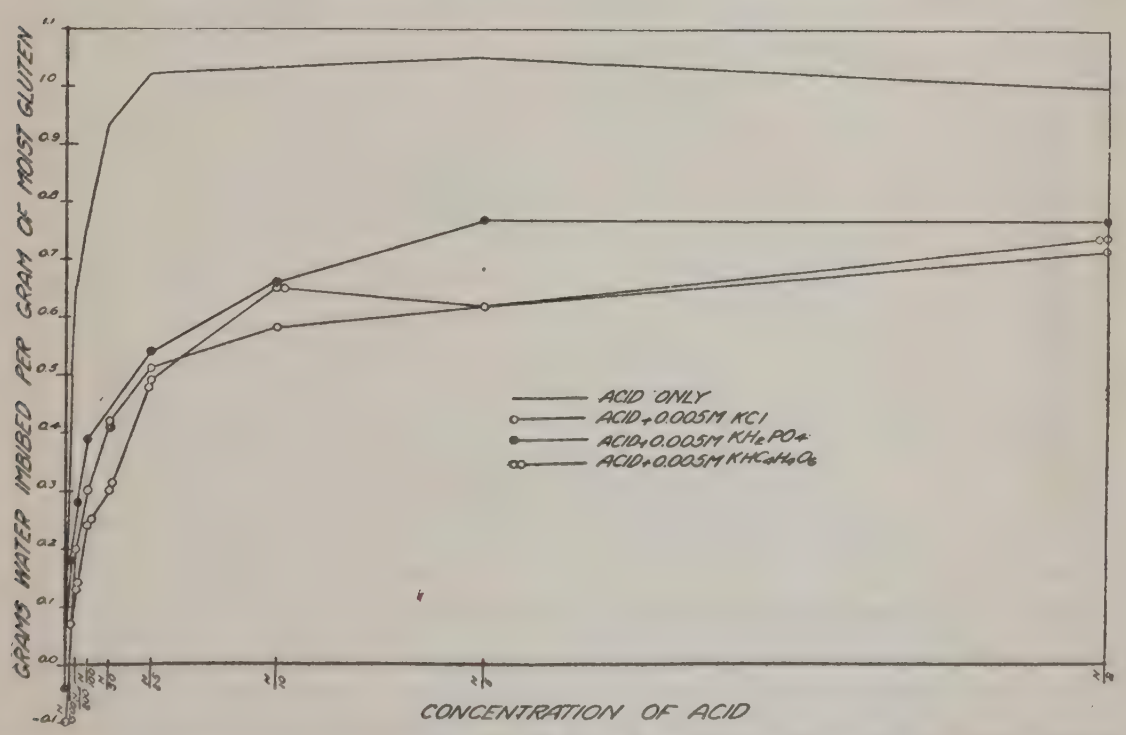

FIG. 9.—Graph showing the imbibition curves for P gluten in acetic acid and in acetic acid plus certain salts.

Expressing these figures in the conventional form of grams of water imbibed per gram of moist gluten and using the patent flour P as our standard, we find that—

C showed an excess of 0.015 gm. of water per gram of moist gluten over P.
W_1 showed an excess of 0.039 gm. of water per gram of moist gluten over P.
W_2 showed a deficiency of 0.026 gm. of water per gram of moist gluten over P.
W_3 showed an excess of 0.137 gm. of water per gram of moist gluten over P.

The figures for C, W_1, and W_2 are certainly within experimental error and as such can have no significance; we must therefore conclude that

these three glutens, although differing widely in "quality" and in physical properties from P, were hydrated to almost exactly the same extent as was P. However, the difference in moisture content of W_3 gluten is probably significant. It is necessary, therefore, to consider what effect this increased moisture content should have in the experiments in Tables I to V, provided that the gluten had originally possessed the same physical properties as P. W_3 contains 7.28 per cent of dry gluten. The dry gluten of P composes 33.15 per cent of the wet gluten; therefore the wet gluten of W_3 should weigh 21.96 gm. at the same hydration as the gluten of P. It actually did weigh 25.45 gm., or an excess of 3.49 gm. of water based on a weight of 21.96 gm. of moist gluten, this indicating an imbibition of 0.16 gm. of water per gram of moist gluten (of P quality). This amount is entirely too small to account

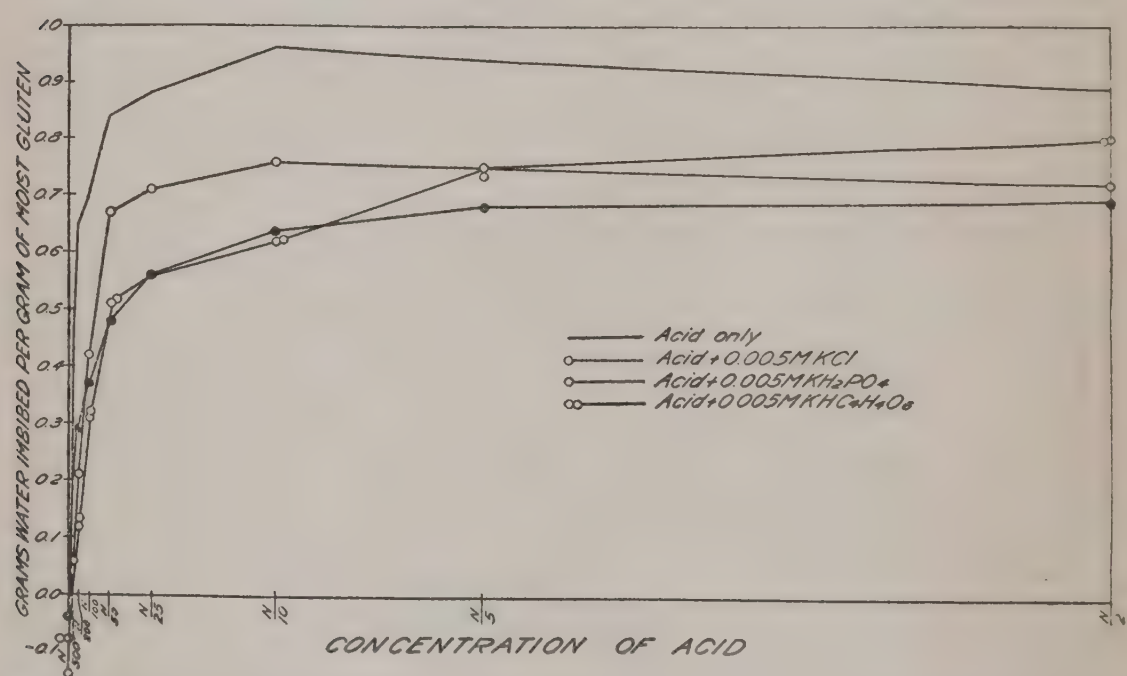

FIG. 10.—Graph showing the imbibition curves for C gluten in acetic acid and in acetic acid plus certain salts.

for the very marked differences in the physical properties of the two glutens and the only conclusion which remains possible is that there is an inherent difference in the glutens from strong and weak flours, that the colloidal properties of the glutens from the different flours are not identical and would not be identical even if the flours had the same salt and acid content.

These data are substantiated by the moist and dry gluten figures presented by Upson and Calvin, although these authors, as noted above, expressed the opinion that the difference between a strong and a weak gluten was due to its degree of hydration. Under Table XII on page 23 of their bulletin (*15*) are given the percentages of wet and dry gluten from several different mill streams of flour. Using carbon-dioxid-free water as the washing agent, they give the percentages of wet and dry gluten as follows:

Flour.	Wet gluten.	Dry gluten.
	Per cent.	*Per cent.*
First middlings	23.5	7.1
Third middlings	27.8	8.7
Fifth middlings	33.7	11.2
Seventh middlings	36.01	12.4

By making the same calculations on these different samples of gluten as was done on our own samples we have the results given in Table VIII.

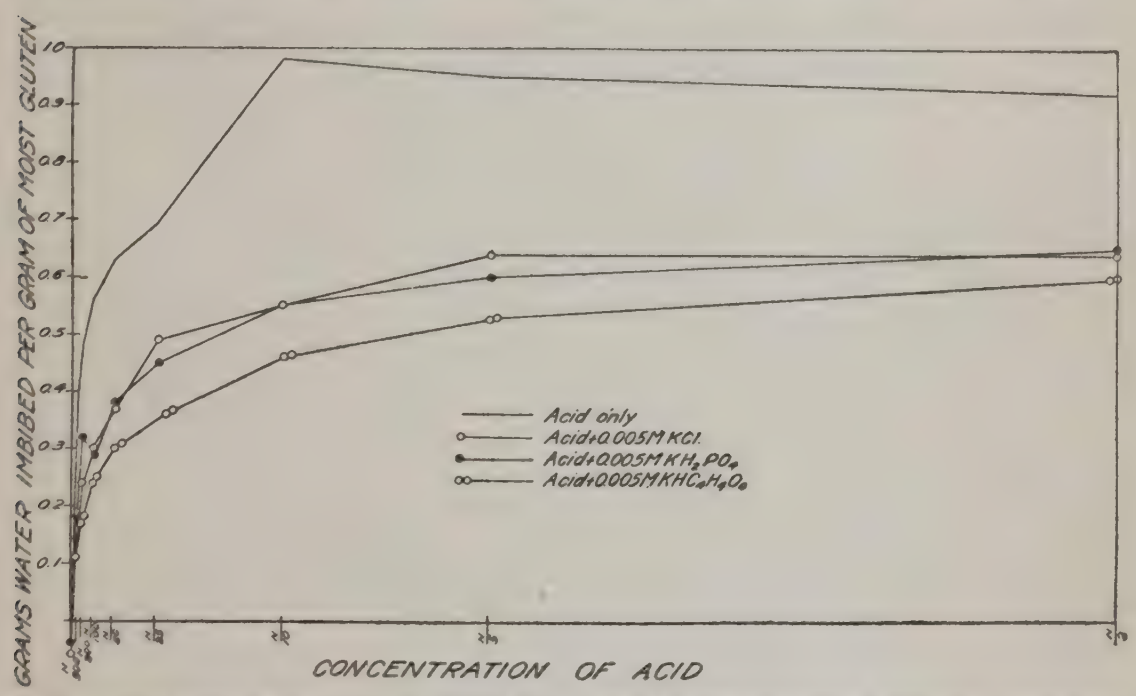

FIG. 11.—Graph showing the imbibition curves for W_3 gluten in acetic acid and in acetic acid plus certain salts.

TABLE VIII.—*Percentage of water in the moist gluten from flours washed with carbon-dioxid-free water*

Flour.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if glutens were hydrated equal to first middlings.	Difference of actual from hypothetical water content.
First middlings	69.8	30.2	.....	.....
Third middlings	68.7	31.3	72.34	− 3.64
Fifth middlings	66.7	33.3	76.96	−10.26
Seventh middlings	65.1	34.9	80.66	−15.56

Again expressing these figures in grams of water absorbed per gram of moist gluten and using the first middlings as the standard, we find that—

third middlings shows a deficiency of 0.036 gm. of water per gram of moist gluten over first middlings; fifth middlings shows a deficiency of 0.103 gm. of water per gram

of moist gluten over first middlings; seventh middlings show a deficiency of 0.156 gm. of water per gram of moist gluten over first middlings.

When a 0.5 per cent sodium-chlorid solution was used as the washing agent, Upson and Calvin obtained wet and dry gluten figures which, when used for calculation, give the results of Table IX.

TABLE IX.—*Percentage of water in moist gluten from flours washed with a 0.5 per cent sodium-chlorid solution*

Flour.	Wet gluten.	Dry gluten.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if glutens were hydrated equal to first middlings.	Difference of actual from hypothetical water content.
First middlings	32.1	9.4	70.7	29.3	.....	.....
Third middlings	34.5	10.3	70.1	29.9	72.2	−2.1
Fifth middlings	37.5	11.2	70.1	29.9	72.2	−2.1
Seventh middlings	38.6	12.0	68.9	31.1	75.0	−6.1

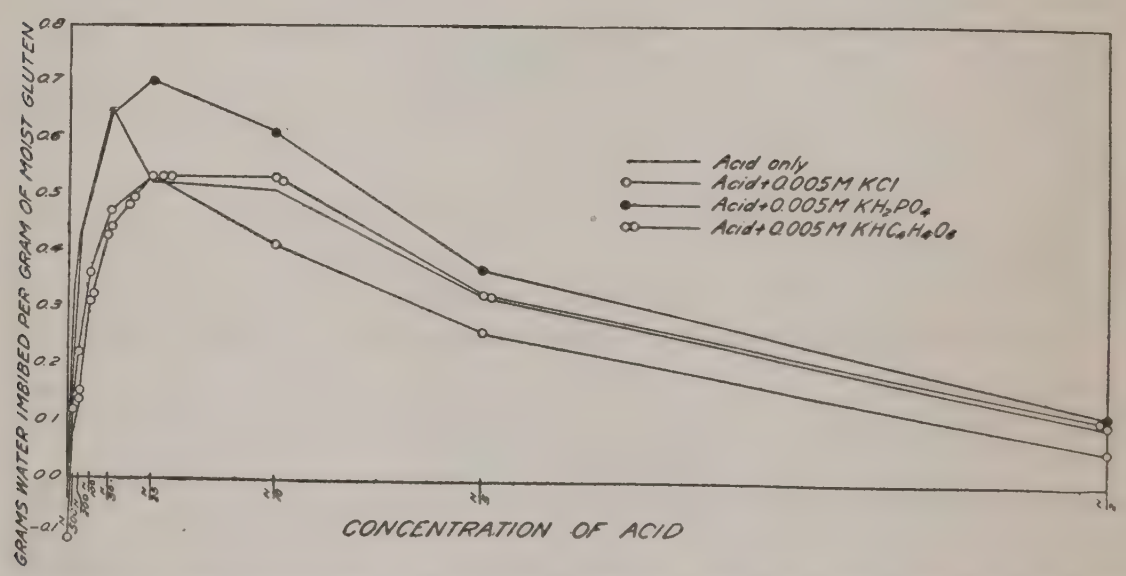

FIG. 12.—Graph showing the imbibition curves for P gluten in oxalic acid and in oxalic acid plus certain salts.

These figures, expressed again in grams of water absorbed per gram of moist gluten with the first middlings as the standard, give the following:

Third middlings shows a deficiency of 0.021 gm. of water per gram of moist gluten over first middlings; fifth middlings shows a deficiency of 0.021 gm. of water per gram of moist gluten over first middlings; seventh middlings shows a deficiency of 0.061 gm. of water per gram of moist gluten over first middlings.

Upson and Calvin found that the seventh middlings gave results similar to a "low-grade" flour the gluten from which, according to their theory, we should expect to find hydrated to a greater degree than that from the first middlings. Such, however, is not the case. In both distilled water and in 0.5 per cent sodium chlorid the "low-grade" gluten

appears to be less hydrated than is the strong gluten. To be sure, four of the six differences are probably within the experimental error, but it is possibly significant that each of the six determinations shows a negative sign. Thus, their own data refute their idea that quality of gluten is regulated by the degree of imbibition and upholds our contention that quality of gluten is not determined by the amount of acids and salts in

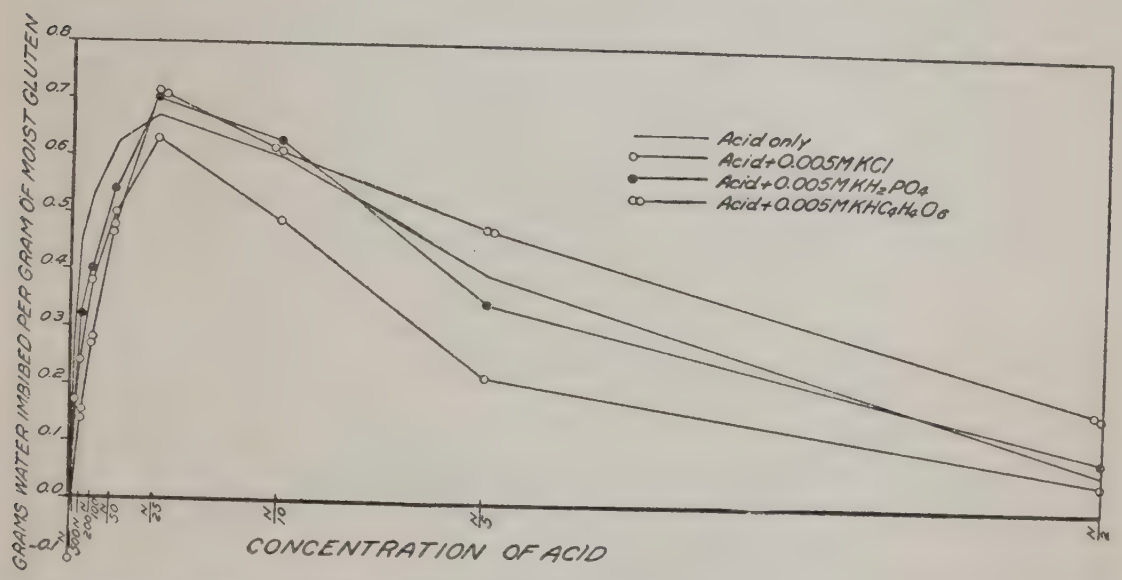

FIG. 13.—Graph showing the imbibition curves for C gluten in oxalic acid and in oxalic acid plus certain salts.

the flour, but by the physico-chemical nature of the colloids comprising the gluten.

Again, these authors list data for a "patent" and a "low-grade" flour in Table XI of their bulletin. These data when recalculated give the following figures:

Flour.	Water in wet gluten.	Dry gluten in wet gluten.	Calculated water content if low-grade gluten were equal to "patent."	Difference between actual and hypothetical water content.
Patent	67.9	32.1	.....	.....
Low-grade	67.5	32.5	68.75	−1.25

The low-grade shows a deficiency of 0.0125 gm. per gram of moist gluten over the patent gluten. This result is within the experimental error of the "patent" grade, but certainly does not support their theory as to the cause of strong and weak glutens.

We have devoted considerable space to the theory of Upson and Calvin because of its important bearing in a study such as we have made, and we believe that we have presented sufficient evidence to show that it has no supporting evidence either in our own work or in any of their publications.

On the contrary, the data in both our tables and in their own are such as to prove the fallacy of their contention and to cause the theory to be definitely discarded.

Guthrie (5) concluded that the property of absorbing water, which a flour possesses, was dependent on the physical nature of the gluten present in the flour rather than upon the absolute quantity of the gluten. He suggests that this physical difference may be due to the relative proportions of glutenin and gliadin in the gluten, inasmuch as he found that dry glutenin will absorb nearly twice as much water as will dry gliadin. However, when his data are recalculated it is at once apparent that the moist gluten from the flours milled from "good bread wheats" were hydrated to almost exactly the same extent as were those from the weak flours, the glutens from the two flours of the former class of the 1896 crop

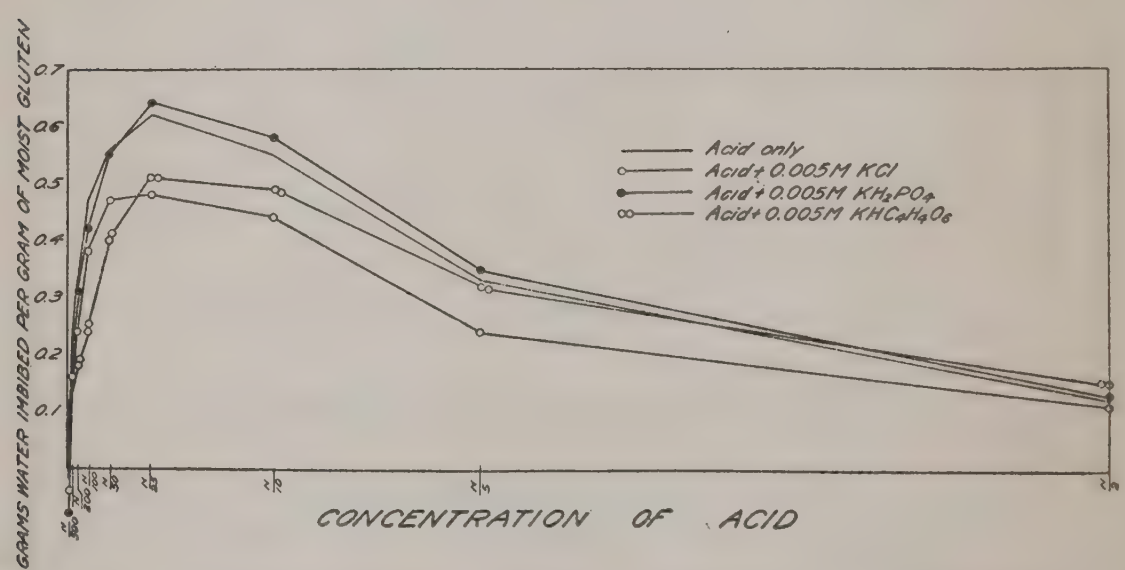

FIG. 14.—Graph showing the imbibition curves for W_3 gluten in oxalic acid and in oxalic acid plus certain salts.

containing 68.19 and 68.53 per cent of water and those from the weak flours 66.44 and 69.10 per cent. Similarly, for the 1895 flours, the strong flour gave 65.58 per cent of water in the moist gluten, while the glutens from three weaker flours contained 63.37, 64.80, and 64.51 per cent of water, respectively. There are certainly no differences in either set of figures large enough to account for the wide differences which were observed in the physical properties of the glutens. Neither do these figures tend to support Guthrie's conclusion that strength is regulated by the glutenin to gliadin ratio.

Turning now to our own data as given in Tables I to IV and in figures 1 to 5, inclusive, we find two noteworthy differences between the strong and weak glutens. These are (a) rate of hydration and (b) maximum capacity for hydration.

RATE OF HYDRATION

It will be observed that the different glutens, prepared and treated in exactly the same manner and for exactly the same interval of time, differ widely in their rate of hydration. For example, in Table I it is shown that 1 gm. of moist gluten from P flour in *N/50* lactic acid imbibed 1.10 gm. of water, while W_1 gluten absorbed only 0.47 gm.; in *N/500* acid the figures are, respectively, 0.63 and 0.12 gm. These examples are typical of other experiments in the tables, and when the graphs are inspected, they are so convincing that only one conclusion seems possible—that is,

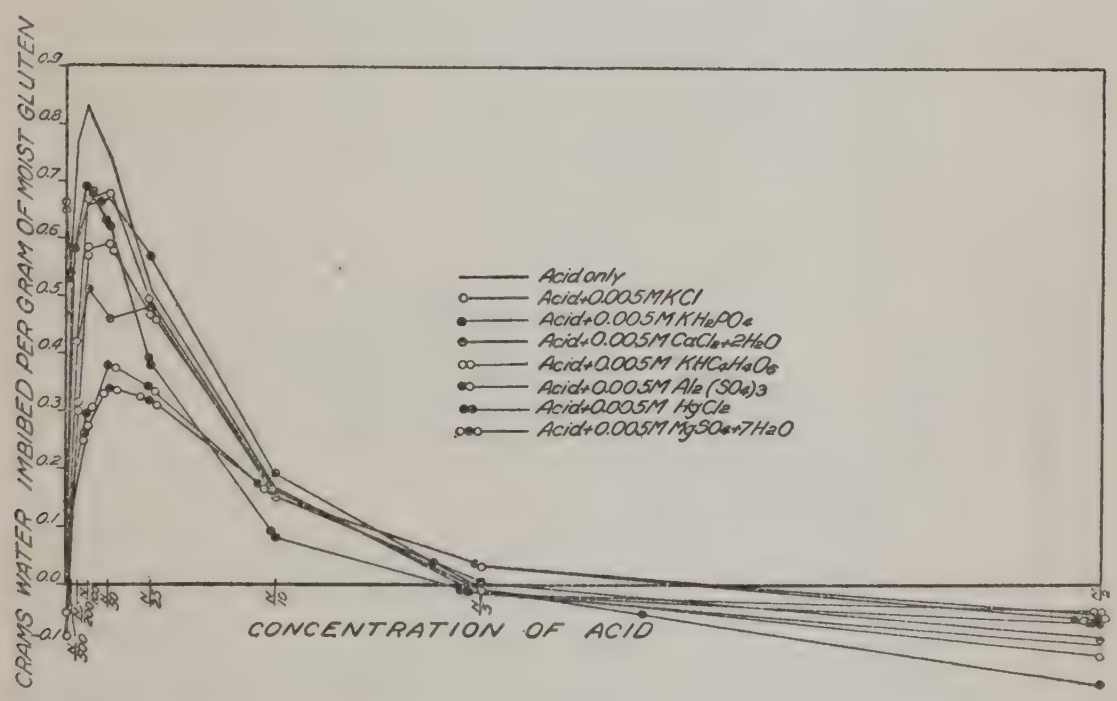

FIG. 15.—Graph showing the imbibition curves for P gluten in hydrochloric acid and in hydrochloric acid plus certain salts.

that a weak gluten has a much lower rate of hydration than a strong gluten.

MAXIMUM CAPACITY FOR HYDRATION

There is, moreover, a marked difference in the maximum degree of hydration of the different glutens. In preliminary experiments it was found that disks of P gluten would retain their coherency and plasticity for as long as two hours in the different concentrations of lactic acid and still be so cohesive that they could be easily removed from the acid solutions by means of small forceps, although they had swelled to three or four times their former size and had imbibed as much as 2.22 gm. of water per gram of moist gluten.

On the other hand, the weak glutens W_1, W_2, and W_3 became so badly dispersed when immersed in the concentrations of acids causing maximum imbibition that in many instances they could not be collected for weighing in even so short a time as one hour, although even at this point

the weight of water absorbed must have been far below the quantity which the P gluten could imbibe and still remain coherent. We are forced to conclude, therefore, that not only does the weak gluten have a lower rate of imbibition than the strong gluten, but that it also has a much lower maximum hydration capacity; or, in other words, a gluten from a "weak" flour changes from a gel to a sol at a much lower degree of hydration than does gluten from a strong flour.

In short, the difference between a strong and a weak gluten is that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoid gels, and that of a colloidal gel in which these properties are much less marked.

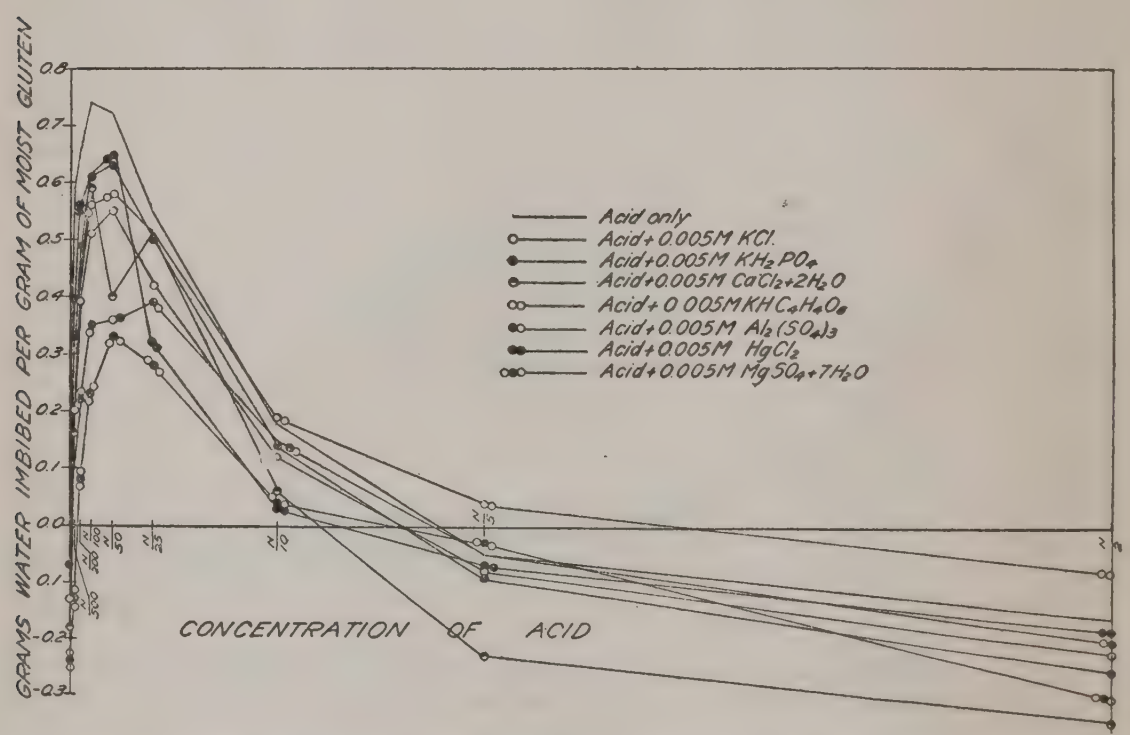

FIG. 16.—Graph showing the imbibition curves for C gluten in hydrochloric acid and in hydrochloric acid plus certain salts.

COMPARATIVE MEASUREMENTS OF THE HYDRATION CAPACITY OF THE DIFFERENT GLUTENS IN VARIOUS CONCENTRATIONS OF LACTIC, ACETIC, PHOSPHORIC, OXALIC, AND HYDROCHLORIC ACIDS

It will be observed that the curves in figures 1, 2, and 3 are very different in form from those of figures 4 and 5. Upson and Calvin (*14*) observed similar differences between the curves for lactic and acetic acids and that for hydrochloric acid.

Both hydrochloric and oxalic acids are highly ionized when compared with the other acids,[1] although phosphoric acid has a dissociation con-

[1] Abbott and Bray (*1, p. 760*) give the following ionization constants: Ortho-phosphoric acid ($H_2PO_4^- + H^+$) 1.1×10^{-2}, ($HPO_4^- + H^+$) 1.95×10^{-7}, acetic acid 1.8×10^{-5}, and boric acid 1.7×10^{-9}. They give the constant for hydrochloric acid as 6×10^{-1}. This is possibly a typographical error, for Noyes (*12, p. 860*) gives the ionization constant for hydrochloric acid as 1, with those for acetic and phosphoric acids of the same value as given by Abbott and Bray. Landolt-Börnstein (*11, p. 1147*) give constants for oxalic and lactic acids as 3.8×10^{-2} and 1.38×10^{-4}, respectively.

stant more nearly like that of oxalic than like those of acetic or lactic acids.

At first glance, leaving the phosphoric-acid graphs out of consideration, it would appear that the amount of imbibition might be regulated solely by the hydrogen-ion concentration, but when we take into consideration the data for both phosphoric and boric acids, one of which is a relatively strong acid but which nevertheless produces the form of imbibition curve typical of much weaker acids and the other a very weak acid which produces no appreciable change in the degree of imbibition of the glutens, it becomes more and more improbable that the hydrogen-ion concentration is the only factor involved. Fischer (*4, p. 44*) has already come to a similar conclusion while studying the imbibition of water by animal proteins or tissues in solutions of acids, for he found that a "strong"

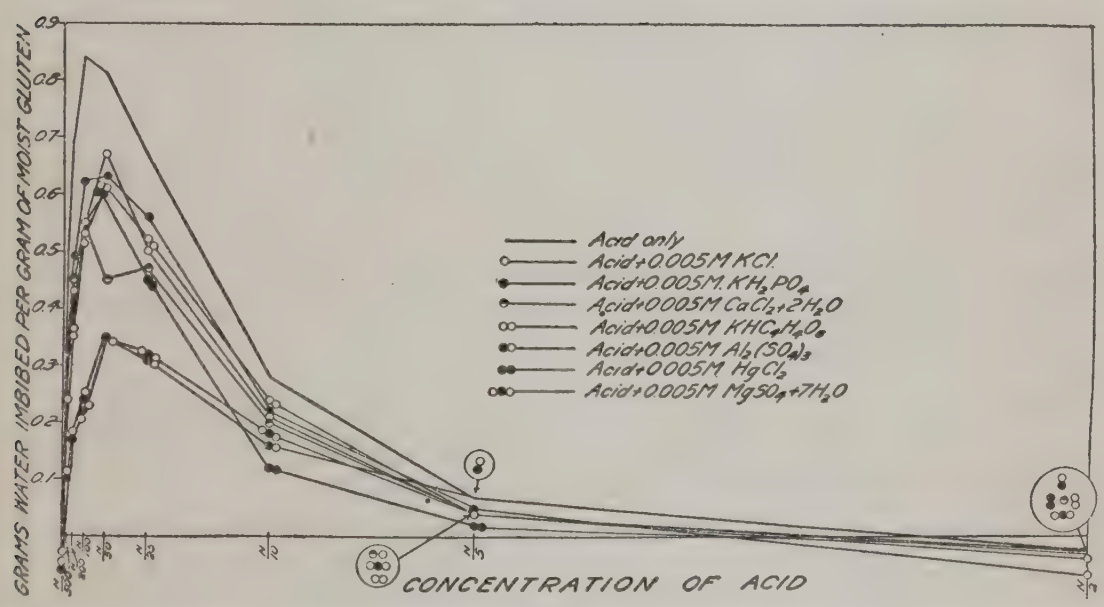

FIG. 17.—Graph showing the imbibition curves for W_3 gluten in hydrochloric acid and in hydrochloric acid plus certain salts.

acid (hydrochloric) stands at the top of the list, another (sulphuric) stands at the very bottom, while a series of "weak" organic acids are found between.

Jessen-Hansen (*10*) has made an extensive study as to the relationship between the hydrogen-ion concentration of the flour and the volume and quality of the resulting loaf. He finds that there is an optimum hydrogen-ion concentration of about $P = {}_{11}5$. For strong flours the optimum may slightly exceed this, and for poor flours it should be somewhat less. Whether or not the beneficial effects of this optimum hydrogen-ion concentration are due to the influence of the hydrogen ion on water imbibition by the gluten or to the securing of the proper reaction of the medium for the optimum growth of the yeast and zymase activity is a problem for further investigation. It is probable, however, that it is the latter factors which are principally influenced.

It is impossible to decide from the existing data the exact factors governing the imbibition of water by colloidal gels in acid solutions, but while the hydrogen ion undoubtedly plays an important rôle, it is equally probable that the undissociated molecule and the anion also strongly influence the degree of imbibition. In phosphoric acid the phosphate ions would be strongly adsorbed by the colloid, and such adsorption may play a great enough rôle in increasing imbibition to offset any depressing influence due to the greater concentration of the hydrogen ions.

However, there can be no doubt, after an inspection of figures 1 to 3, that there is an inherent difference in the colloidal properties of the different glutens. With lactic and acetic acids the P gluten rises more abruptly and to a higher maximum than with any of the others. The weaker glutens, especially W_2 and W_1, have a much flatter curve.

In oxalic and hydrochloric acids (fig. 5, 6) there is a much greater degree of uniformity between the different glutens, but we believe that even here certain differences may be detected, for the curves for the W_2 and W_1 glutens are again much flatter and have lower maximums than the P or C glutens.

ANTAGONISTIC ACTION OF SALTS UPON THE IMBIBITION OF WATER BY THE VARIOUS GLUTENS IN THE PRESENCE OF HYDROCHLORIC, OXALIC, LACTIC, AND ACETIC ACIDS

Tables I to IV and figures 6 to 17 show the imbibition data for the various glutens when certain salts in 0.005 molar concentration were added to the various concentrations of the acids.

In nearly every instance the addition of the salts decreases the amount of imbibition and also changes the form of the hydration curve so that a higher concentration of acid is necessary to produce maximum imbibition. There is also a noticeable difference in the behavior of the different glutens. Certain of these differences are recorded in Table X, where the acid concentrations are recorded for the various solutions of lactic and acetic acids at the maximum imbibition of the different glutens. The corresponding figures for oxalic and hydrochloric acids are not included, inasmuch as the curves for these "stronger" acids are much more nearly identical. It is of interest to note that whereas $N/5$ acid is the highest concentration of acid causing maximum imbibition in the acids alone, when salts are added there are 15 instances where maximum imbibition is not yet reached at an acid concentration of $N/2$. The behavior of the different glutens in these solutions is a strong argument for the hypothesis that the physico-chemical properties of the glutens are not identical. This is especially true for the curves for calcium chlorid, magnesium chlorid, and aluminium sulphate. As we pass from P gluten to C gluten and finally to W_3 gluten, these curves show less tendency to reach maximums and then decline. The curve for lactic acid plus calcium chlorid is particularly striking.

TABLE X.—*Concentration of the lactic and acetic acids at the maximum point on the various imbibition curves*

Solution.	Lactic acid.			Acetic acid.		
	P	C	W_3	P	C	W_3
Acid alone	N/10	N/25	N/10	N/5	N/10	N/10
Acid+potassium chlorid	N/5	N/10	N/5	> N/2	N/10	> N/2
Acid+potassium phosphate	> N/2	N/5	N/10	N/5	> N/2	> N/2
Acid+potassium tartrate	> N/2	N/5	N/5	> N/2	> N/2	> N/2
Acid+calcium chlorid	N/5	> N/2	> N/2	.....	.....	.....
Acid+magnesium sulphate	N/5	N/5	> N/2	.....	.....	.....
Acid+mercuric chlorid	< N/500	< N/500	< N/500	.....	.....	.....
Acid+aluminium sulphate	> N/2	> N/2	> N/2	.....	.....	.....

The imbibition curves in the presence of lactic acid plus mercuric chlorid are the reverse form from the curves in the presence of the other salts. Here maximum imbibition takes place in the 0.005 molar solution of mercuric chlorid, and when acid is added, the imbibition rapidly decreases. The more inferior the gluten the lower is this minimum imbibition. When, however, the lactic acid is replaced by hydrochloric acid, maximum imbibition does not take place in the 0.005 molar solution of mercuric chlorid alone, but in solutions containing both the mercuric chlorid and hydrochloric acid. The imbibition curves here are comparable in form to those for the other salts. Just what factors cause this reversal of form of the curves in the two instances is uncertain, and the subject was not further investigated, inasmuch as it was thought to be of more theoretical than of practical interest.

TABLE XI.—*Average imbibition of the different glutens in the various solutions of acids and of acids plus salts*

Solution.	Lactic acid.			Acetic acid.			Oxalic acid.			Hydrochloric acid.		
	P	C	W_3	P	C	W_3	P	C	W_3	P	C	W_3
Acid alone[1]	1.01	0.85	0.82	0.86	0.78	0.70	0.41	0.45	0.40	0.43	0.40	0.48
Acid+potassium chlorid	.69	.62	.56	.42	.53	.41	.30	.33	.31	.32	.25	.31
Acid+potassium phosphate	.64	.69	.62	.50	.49	.42	.48	.39	.39	.35	.29	.35
Acid+potassium tartrate	.65	.67	.52	.40	.46	.34	.30	.36	.30	.27	.29	.30
Acid+calcium chlorid	.52	.52	.50	.....	.....	.....	.....	.....	.....	.26	.19	.29
Acid+magnesium sulphate	.32	.27	.27	.....	.....	.....	.....	.....	.....	.15	.06	.16
Acid+mercuric chlorid	.36	.33	.15	.....	.....	.....	.....	.....	.....	.34	.28	.30
Acid+aluminium sulphate	.33	.33	.27	.....	.....	.....	.....	.....	.....	.17	.16	.17

[1] The figures on W_1 and W_2 glutens were, respectively; Lactic acid alone, 0.65 and 0.49; acetic acid alone, 0.32 and 0.48; oxalic acid alone, 0.38 and 0.39; and hydrochloric acid alone, 0.30 and 0.31.

Table XI shows the average imbibition of the different glutens in the different acids and salts. Each of these figures represents the average point of an entire curve, and as such represents from 24 to 56 individual determinations of the weight of water imbibed. The averages of such a large number of determinations certainly ought to have significance.

Here again the differences in the stronger acids do not appear very striking. Those for lactic acid are, however, very consistent. In every instance the W_3 gluten shows lower imbibition than P gluten, and the antagonistic action of salts on imbibition by the P gluten is very much more pronounced than with either C or W_3 gluten.

There is a certain similarity between the curves for the P gluten and salt and those for the weaker glutens in acid alone. There is, however, no necessary correlation, for a flat, slowly rising curve means only that the imbibing power of the gluten is low. In one instance this is due to the antagonistic action of the salts upon the action of the acid and in the other instance to an inherently weaker tendency on the part of the colloid to imbibe water. That these differences actually do exist in the two instances is shown by the fact that the presence of salts in the acid solution causes the gluten to retain its coherence and become more firm and elastic than controls in the same concentration of acid lacking the salts. This does not hold for the weak glutens with the flat imbibition curves, for these lose their coherence, become weak and inelastic and disperse at a much lower degree of hydration than do those glutens whose curves rise sharply.

We can therefore definitely state that a weak gluten does not owe its "weakness", nor its imbibition curve its "flatness", to either the acid or the salt content of the flour from which it is derived, but rather to the fact that a weak gluten has inherently inferior colloidal properties.

BAKING TESTS AND FLOUR ANALYSES

In the foregoing experiments and discussion, considerable attention has been directed to the effect of acids and salts upon glutens prepared from strong and weak flours, and we believe that it has been clearly shown that the determining factor in flour strength is not the concentration of soluble acids and salts which are present in the flour.

Further evidence, however, that "quality" in flours is not determined by the soluble-acid and salt content is again presented in Table VI. From the data therein given, it is to be observed that the patent flour ranks first in baking quality. It absorbs more water in the doughing process, producing a dough much more coherent and elastic, as is shown by the maximum expansion of the dough during the process of fermentation, and produces a loaf of the largest size and of the best texture.

It will also be noted that the patent flour is somewhat lower in its total and soluble ash content, and electrical conductivity. However, the differences between the patent flour, the flour with the strong gluten, and the W_3 flour, the flour with much weaker gluten, give values for ash on dry flour, soluble ash, and specific conductivity of the flour extract, all of which are within experimental error of each other, an observation which confirms the previously expressed idea that strength or weakness of gluten is due to the colloidal condition of the flour proteins, and is not

determined to any great extent by the inorganic material present in the flour. The soluble ash and specific conductivity are almost parallel to each other in every case. This was to be expected, since the determinations were made on identical flour extracts.

The bread baked from the weak flours was of poor texture. In each case the dough lacked the coherency and elasticity necessary to produce "large well-piled" loaves. This is without doubt partially but not entirely accounted for by the lower gluten content of these flours.

WHAT DETERMINES THE PHYSICAL STATE OF THE GLUTEN

Upson and Calvin (*14, 15*) believe the differences between strong and weak glutens to be due to acids or salts in the flour, but it has been shown earlier in this paper that there is no evidence for such an assumption.

Wood (*17*) likewise states that the physical properties of the gluten are due—

> to varying concentrations of acid and soluble salts in the natural surroundings of the gluten.

This would appear to be identical with the view of Upson and Calvin, but while Upson and Calvin believe that the controlling factors are present in the flour, Wood (*17, p. 274*) believes that the character of the gluten is altered at the time when it is laid down in the wheat kernel.

> It must be decided at what stage the acids and salts influence the gluten so as to mpress upon it the physical characters which decide the physical character of the-flour. I take it that this must occur when the endosperm is being formed, at which time the grain contains much more water than when it is ready to grind.

Wood and Hardy (*17*) suggest that each particle in a gluten hydrosol is surrounded by an electric double layer and that the—

> tenacity, ductility, and water-content of a solid mass of moist gluten depends upon the total or partial disappearance of these electric double layers, and the reappear ance of what is otherwise obscured by them, namely, the adhesion or "idio attraction," as Graham called it, of the colloid particles for each other, which makes them cohere when they come together—the most complete coagulation, i. e., mechanically the densest and most coherent coagulum being formed at the isoelectric point.

However, in all of the papers by Wood or Hardy the assumption is apparently made that at the isoelectric point all glutens are identical in physico-chemical properties. This we do not believe to be the case, for if all glutens were identical at the isoelectric point and the degree of hydration, etc., were regulated by the presence or absence of electric double layers around the colloidal particles, we should expect to find approximately the same maximum hydration capacity for each gluten preparation, although the maximum point on the hydration curves might be reached at different concentrations of acids. We have already shown that the glutens from different sources differ in rate of hydration and in their maximum hydration capacity, and we believe that these

factors show that even at the isoelectric point there would be wide differences in their physical properties. It appears extremely probable that the actual cause of the physico-chemical differences between strong and weak glutens may be due to the form in which the protein is laid down in the endosperm. It is entirely within reason to suppose that the gluten may, under certain environmental conditions, be deposited as uniformly-sized particles with the characteristics of true emulsoids, while under different environmental conditions a part of the gluten may be deposited in this form and another part in a semicrystalloidal form. In other words, we postulate that the particles in a weak gluten are on the average nearer the boundary line which separates the crystalloidal state of matter from the colloidal state than are the particles comprising a strong gluten. It is the intention of one of us to test this hypothesis in the near future.

SUMMARY

In this paper are presented data showing the increase or decrease of water imbibition caused by immersing weighed disks of gluten from five selected flours in solutions of lactic, acetic, boric, phosphoric, hydrochloric, and oxalic acids of various concentrations, both with and without the addition of 0.005 molar concentrations of certain salts.

Data have also been presented showing different flour analyses such as ash on dry flour, soluble ash, specific conductivity of flour extract, percentage of moist gluten, percentage of dry gluten, percentage of ash in dry gluten, and baking tests.

From a study of these data, the following conclusions have been drawn:

(1) Although the moist glutens from these flours differ widely in "quality" and in physical properties, they are hydrated to almost exactly the same extent.

(2) Gluten from a weak flour has a much lower rate of hydration than gluten from a strong flour.

(3) Gluten from a weak flour has a much lower maximum hydration capacity than gluten from a strong flour, changing from a gel to a sol at a much lower degree of hydration.

(4) Two types of imbibition curves were observed. Dilute solutions of hydrochloric acid and of oxalic acid cause the gluten to rapidly imbibe water, while at slightly stronger concentrations of acid water is actually extracted from the moist gluten. Dilute solutions of lactic, acetic and phosphoric acids cause the gluten to strongly imbibe water but stronger acid solutions only slightly diminish the imbibition. The hydrogen-ion concentration of the acid is not the only factor influencing imbibition, but it is pointed out that the anion and the undissociated molecules, as well as their relative adsorption by the protein, must in all probability be taken into consideration.

(5) Inorganic salts when added to an acid solution lower the relative imbition of gluten placed in such solutions. Glutens from the different flours react differently to the addition of inorganic salts.

(6) The acid and salt contents of the flours are not responsible for the difference between a strong and weak gluten.

(7) The postulation that the different physical conditions observed in glutens derived from different flours are due solely to the presence or absence of an electric double layer around the colloidal particles is not consistent with the facts recorded in this paper. A strong gluten would differ from a weak gluten even at the isoelectric point.

(8) There is an inherent difference in the glutens from the strong and weak flours. The physico-chemical properties of the glutens from the different flours are not identical and would not be identical even if the flours had originally had the same acid and salt content.

(9) The difference between a strong and weak gluten is apparently that between a nearly perfect colloidal gel with highly pronounced physico-chemical properties, such as pertain to emulsoids, and that of a colloidal gel in which these properties are much less marked. It is suggested that such differences may be due to the size of the gluten particles and that at least a part of the particles comprising the weak gluten may lie nearer the boundary between the colloidal and crystalloidal states of matter than is the case with the stronger glutens.

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CHEMISTRY AND HISTOLOGY OF THE GLANDS OF THE COTTON PLANT, WITH NOTES ON THE OCCURRENCE OF SIMILAR GLANDS IN RELATED PLANTS [1]

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INTRODUCTION

The work herein reported forms a portion of a chemical and biological investigation of the cotton plant (*Gossypium* spp.), the purpose of which is to isolate and determine the substance or substances which attract the boll weevil. A previous paper (*17*) [2] discusses the isolation of certain glucosids and the products of their hydrolysis, as well as preliminary studies of an ethereal oil which manifested some attraction for the boll weevil. Both the glucosids and this oil, as well as several other substances, are largely localized in prominent internal glands which are very numerous in nearly all parts of the cotton plant. The main purpose of this paper is to discuss the occurrence, formation, structure, and contents of these glands.

Glands of another type, more properly referred to as "nectaries," also occur in the cotton plant. These are superficial in position and definitely localized. The internal glands have nothing in common with these nectaries save the function of secretion. In certain taxonomic and other literature, however, either or both types are referred to indiscriminately simply as "glands." Therefore, it seems advisable also to discuss briefly in this paper the nature and occurrence of the nectaries, in order to distinguish them clearly from the internal secretory organs, which form the main subject of the present study.

I.—THE INTERNAL GLANDS

The internal gland of cotton consists of an oblate or spheroidal central sac 100 to 300 μ in diameter, filled with a more or less homogenous yellow or brownish secretion, surrounded by an envelope of one or more layers of flattened cells, which in the glands exposed to light contain a red pigment.

DISTRIBUTION OF INTERNAL GLANDS

Because of their dark color, which renders them plainly evident beneath the epidermis of the green stem or palisade layer of the foliage, and because of the supposed nature of their content, the internal glands have been

[1] Second paper of a series on the chemistry of the cotton plant, with special reference to Upland cotton.

[2] Reference is made by number (italic) to "Literature cited," p. 434-435.

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variously alluded to as "black glands," "gland-dots," "resin glands," "oil glands," "gossypol glands," etc. They are constantly present and definitely arranged throughout the genus Gossypium, although their prominence varies in different species according to their size, proximity to the surface, depth of pigmentation, and presence or absence of obscuring hairs or tomentum.

Within the seed (Pl. 42, A, C) the glands are found directly beneath the palisade layer, into which they often project, causing a shortening of the palisade cells, but usually no bulging of the surface. Here they are oblate-spheroidal in form, with long axis perpendicular to the cotyledon surfaces. Their long axes frequently exceed half the width of the cotyledon—that is, 100 to 200 μ. Smaller glands are also found in the cortex of the radicle, covered by a few parenchymal layers.

As the seedling develops, glands are formed profusely in the primary cortex of the hypocotyl (Pl. 43, A, B; 44, A, B) and sparingly in that of the radicle (Pl. 45, A, B). In the former they are nearly globular, and in the latter much elongated. In the hypocotyl and the young stem the glands occur very close to the epidermis, and often push it outward in their development, appearing then like small, dark warts. Their formation keeps pace with the development of the foliage; in the unfolded cotyledons and true leaves they are located beneath the palisade layer in the centers of small areas bounded by the anastomosing veins. Stipules, bracteoles, and also the calyx and corolla (Pl. 46, A, *a*, *b*) are glandulate, the arrangement of glands being in general similar to that of the foliage, though not all netted areas possess glands. Glands occur also within the tissues of the anther and the staminal column (Pl. 46, A, *c*, *d*). The principal veins of the bracteoles frequently appear nonglandulate, in marked contradistinction to the areas between them; on inspection with a hand lens, however, small glands may usually be seen upon them. The veins of the calyx are nearly destitute of glands; these veins are set closely together, and the glands between them therefore seem to run in parallel lines. Glands occur plentifully upon the style, in rows between and below the stigmas. The boll possesses relatively very large glands; in *G. hirsutum* L. (Pl. 46, B) they are beneath several cell layers, and are accordingly less conspicuous than in *G. barbadense* L., where they are close to the surface, which is pitted above them. The glands of the green parts are very nearly spherical in form.

The secondary cortex also may contain glands of a slightly different type (Pl. 47, A). These always occur within the expanded ends of the medullary rays. The formation of glands in the secondary cortex would seem to be influenced by the stimulus of light, They are rarely formed in the secondary cortex of the stem (Pl. 47, B) and here apparently only where the outer tissues have become considerably suberized and opaque. They occur, however, very plentifully in the root; several are usually found in each phloem ray.

No glands are found in the xylem or phloem proper nor in the seed coats.

DEVELOPMENT OF INTERNAL GLANDS

Internal glands, with the exception of those of the secondary cortex, are developed from certain cells of the ground meristem. Those of the seed are formed coincidentally with the development of the tissues of the embryo, which are formed from the endosperm soon after fertilization takes place. The process of gland development in all parts of the plant, except the secondary cortex, is essentially the same. Within the rapidly dividing tissue of the ground meristem a well-defined circle of cells marks the boundary of the developing gland; the cells within this circle are more or less concentrically placed (Pl. 48, A). A marked change of content takes place in the central cells; the protoplasm becomes vacuolate and arranged in strands and is converted into a yellowish oily-appearing substance (Pl. 48, B, C). These changes are accompanied by rapid swelling of the cells concerned, some of which are crushed and obliterated in the process, while the peripheral layers become much flattened. The nuclei of the swelling cells enlarge and soon degenerate and disappear. The walls of the swollen cells usually are dissolved and disappear rapidly, leaving the gland as a large central cavity surrounded by layers of the flattened cells. In the seed glands (Pl. 42, C), however, the interior walls never wholly disappear, but vestiges, easily dissolved in water and probably of a mucilaginous nature, remain. The writers regard the process of gland formation as truly lysigenous, for traces of the secretion can be observed in the unbroken cells, and not, as the more common schizogenous glands, first in the intercellular spaces, as figured by Tschirch (*15, p. 1095–1268*).

While the secretion is first formed in the central cells, its general solidification in the seed indicates that it may be added by the flattened cells in the encircling layers, which then in their turn may act as secretory cells, as in a schizogenous gland. The presence of this layer, usually characteristic of the latter type of gland, is doubtless the cause for Dumont's (*2*) reference to the glands of *Gossypium* spp. as schizogenous. The cells of the encircling layers retain their nuclei and their walls at length become considerably thickened. In the seed they are somewhat mucilaginized, dissolving partially in water and cuprammonia, but not in alcohol, and giving no well-defined cellulose reactions. In this latter respect, however, they do not differ from the surrounding cell walls, which are not water-soluble.

The development of glands in the secondary cortex is similar, but usually simpler. One or more cells may be involved in the process. Upon their division from the cambium the nuclei quickly disappear, the cells become filled with a dense yellowish oily substance, and enlarge rapidly. When more than one cell is involved, the dissolution of the dividing walls is more rapid than in the glands previously described. The distension of the secretory cells flattens one or two surrounding cell layers into an envelope resembling that of the primary gland.

SECRETIONS OF THE INTERNAL GLANDS

Microscopical investigations of the secretion of the internal gland have shown the presence of a variety of substances and also of differences of content, according as the glands are or are not exposed to the action of light. Most evident are a deep-red pigment and a yellow or brownish oily-appearing substance. The red, or sometimes purple, pigment is deposited in amorphous semisolid or in liquid form within the flattened layers of the glands of illuminated parts. It is most prominent in the glands of the young green parts, and its density accounts for their "black" appearance; the red coloration may be made microscopically evident by crushing the gland, thereby diluting the pigment in cell sap. The coloring of conspicuous red spots on cotton foliage often originates in wounded glands. No red pigment is normally formed in the glands of the seed or secondary cortex. A small amount is usually developed in the glands of the cotyledon when the latter becomes functional as a leaf. Pigment is first formed in the glands of the petals as the latter protrude from the bud.

This red pigment gives the reaction typical of anthocyans. It is soluble in water and alcohol, nearly insoluble in ether, and insoluble in petroleum ether. Acids dissolve it with a brilliant red coloration. Green or blue colors are first formed with alkalis; strongly alkaline solutions are soon decolorized. Basic lead acetate forms a dark-green precipitate. Iron salts cause a blue or purple coloration. The red pigment of the glands is apparently more strongly developed in *G. barbadense* than in *G. hirsutum*. If a young leaf of the former species be slightly crushed, the diffusion of anthocyan may color the whole injured area a brilliant red.

The content of the central chamber of the gland varies from a yellow oily fluid in the young gland to a resinous-red solid substance in the mature gland of the seed. With proper reagents the yellow secretions of the glands exposed to light are seen to differ in character from those of the inner cortex of the stem, root bark, seeds, and the partially developed corolla, which, by their positions, are shielded from the influence of light.

SECRETIONS OF THE GLANDS EXPOSED TO LIGHT

In the large glands of the boll occurs a bright-yellow oily substance in which may be intermixed granular fragments of a dull-yellow or orange color. In the smaller glands of other outer portions of the plant the yellow substance occurs in smaller amount; the solid sometimes predominates. Reactions show the solid, in the main, to be a condensation product of the yellow liquid. The liquid, which flows out in globules as the gland is cut, is nearly insoluble in water and is not readily emulsified in it. It is very soluble in ethyl and methyl alcohol, acetone, chloroform, and ether, forming a bright-yellow solution. It is almost or quite insoluble in petroleum ether and xylene. In most acids

it is wholly or nearly insoluble. In alkalis the globules swell, become orange in color, and dissolve, forming a bright-orange solution which becomes yellow on dilution. A dark-green precipitate is formed by alcoholic ferric chlorid, and an orange or yellow precipitate by lead acetate. These reactions are characteristic of flavones. Quercetin and several of its glucosids have been shown by Perkin (*9*, *10*, *11*) to be present in the cotton plant, and a previous paper (*17*) from this Bureau discusses the isolation of quercetin and two of the glucosids, quercimeritrin and isoquercetin, from the flowers and foliage of the species here considered. The latter glucosid was present only in very small amount. The reactions of these substances correspond well with those of the yellow globules of the glands, and no reactions of flavones have been noted in other portions of the green parts.

SECRETIONS OF GLANDS NOT EXPOSED TO LIGHT

The secretion of the young glands which are not exposed to light also consist of a yellow fluid, with a slightly greenish tinge. In a mature or dried gland it becomes hardened into a reddish-resinous solid. It differs from the secretion of the glands mentioned in the preceding paragraph in forming a greenish emulsion with water and aqueous reagents. It is soluble in alcohol, ether, acetone, and chloroform, but is insoluble in petroleum ether and xylene. Its behavior with alkalis is similar to that of the secretion of the illuminated glands. The chief reaction by which it may be identified within the gland is an intense red coloration induced by concentrated sulphuric acid, which reagent dissolves quercetin and its glucosids with more or less difficulty, forming a yellow solution. This red reaction, first noted by Hanausek (*5*) was cited by Marchlewski (*7*) as a property of gossypol, which Withers and Carruth (*19*) consider to be the toxic agent in cotton seed. This red reaction is characteristic of all stages of the glands of the seed and secondary cortex, but is absent in those of the primary cortex and foliage. Glands of the developing petal at first give the gossypol-red reaction, but before or soon after the petal unfolds they lose this property and react as the glands of the green parts. Synchronously with this change of character occurs the development of anthocyan in the enveloping cell layers. A strip of petal may show all gradations from intense red to yellow reactions with sulphuric acid, according as the glands have been exposed to the light. These changes were clearly marked in blossoms of *G. barbadense*, and were confirmed in field-grown specimens of *G. hirsutum*. No gossypol was recovered from 800 gm. of ground, dried cotton flowers by the method by which Withers and Carruth (*19*) isolate the substance from cottonseed kernels. Their process consists in precipitating gossypol from the ether extract by petroleum ether.

Glands of blossoms of the Upland cotton plant which had been grown in a greenhouse whose roof was so heavily painted as to prevent largely

the passage of light, never lost their gossypol reaction; moreover, little or no anthocyan was found, either in the glands or the petals.

With the unfolding of the cotyledon in the seedling the preformed gossypol undergoes a change; it gives a brownish or greenish emulsion or solution with sulphuric acid. The anthocyan pigments give a similar but less intense red with sulphuric acid; they, moreover, react with acid in any strength, while gossypol develops the red coloration only with concentrations of 80 per cent (volumetric) or more, and the coloration is immediately lost if the gossypol-red solution be diluted below this strength.

Table I, which gives the microchemical reactions of the contents of the gland chambers of parts exposed and not exposed to light, is herewith appended. The more important reactions are compared with those of pure quercetin and quercimeritrin, prepared in this Bureau, and pure gossypol, kindly furnished by Dr. F. E. Carruth, of the North Carolina Experiment Station. Experiments to distinguish quercetin from its glucosids *in situ* have thus far not been successful.

TABLE I.—*Microchemical reactions of the secretions of the internal glands of cotton and of the substances isolated therefrom*

Reagent.	Glands unexposed to light.	Glands exposed to light.	Gossypol.	Quercimeritrin.	Quercetin.
None	Greenish-yellow fluid to reddish-brown solid.	Bright-yellow globules and slightly darker solid.	Yellow crystals	Yellow crystals	Yellow crystals.
Distilled water	Rapidly emulsified, yellow, or olive-green.	Emulsified slightly or not at all.	Insoluble	Insoluble	Insoluble.
Alcohol, absolute	Slowly dissolving yellow	Rapidly dissolving; yellow	Soluble; yellow	Slowly soluble; yellow	
Alcohol, 95 per cent	do	Rather slowly dissolving; yellow.	do	do	
Alcohol, 70 per cent	Very slowly dissolving; yellow.	Very slowly dissolving; yellow.	Slowly soluble; yellow	Very slowly soluble; yellow	
Alcohol, 50 per cent	Slowly emulsified and slightly dissolving.	Dissolving little or not at all			
Methyl alcohol	Rather rapidly dissolving	Rapidly dissolving			
Amyl alcohol	Slowly dissolving	Rather rapidly dissolving	Readily soluble		
Ether	do	Rapidly dissolving	Rapidly soluble	Nearly or quite insoluble	Nearly or quite insoluble.
Chloroform	do	do	do		
Acetone	Rather rapidly dissolving	Instantly dissolving	do	Rapidly soluble	
Acetic ether	Slowly dissolving	Rapidly dissolving	do		
Petroleum ether	Not dissolving	Not dissolving	Not soluble	Not soluble	
Xylene	do	do	do	do	
Carbon disulphid	Rather rapidly dissolving	Rather rapidly dissolving	Readily soluble		Slowly soluble.
Carbon tetrachlorid	Little or not at all dissolving	do	do		Do.
Aniline	Very rapidly dissolving; bright yellow.	Rapidly dissolving; orange to yellow.	Immediately soluble; bright yellow.	Slowly soluble	
Glycerin	Very slowly dissolving	Slowly dissolving	Insoluble cold; soluble hot		
Chloral hydrate	Emulsified, slowly dissolving; greenish yellow.	Emulsified, slowly dissolving; yellow.	Slightly soluble	Slowly soluble	
Cottonseed oil	Dissolving on heating	Dissolving at about 50° C.	Very slowly cold; readily at 60° C.		
Volatile oil distilled from cotton plants.	Slowly dissolving cold; less slowly with heat. Practically all dissolved at 79° C. in 5 minutes (seed).	Readily dissolving in cold. Anthocyans dissolve with heat at 50° to 60° C.	Readily soluble in cold; greenish yellow.	Readily soluble in cold. Yellow.	Slowly soluble in cold.
Acid, acetic (concentrated)	Slowly dissolving; yellow	Quite rapidly dissolving	Very slowly soluble	Slightly soluble	Slightly soluble.
Acid, hydrochloric (concentrated)	Emulsified, olive or yellow-green.	Not dissolving	Insoluble	Slowly soluble (?)	
Acid, nitric (concentrated)	Slowly dissolving; orange	do	Nearly insoluble		
Acid, sulphuric (concentrated)	Immediatly dissolving; brilliant red.	Dissolving; yellow to orange	Immediately soluble; brilliant red.	Rapidly soluble; bright yellow.	Rapidly soluble; yellow to orange.
Acid, sulphuric (concentrated), 80 per cent.	Rapidly dissolving; brilliant red.	Slowly, or not at all, dissolving; yellow to orange.	do		
Acid, sulphuric (concentrated), 65 per cent.	Emulsified; brown	Not dissolving	Slightly soluble; pink		
Acid, sulphuric (concentrated), 50 per cent.	do	do	Insoluble, hot or cold		

TABLE I.—*Microchemical reactions of the secretions of the internal glands of cotton and of the substances isolated therefrom*—Continued

Reagent.	Glands unexposed to light.	Glands exposed to light.	Gossypol.	Quercimeritrin.	Quercetin.
Sulphuric acid plus absolute alcohol, equal parts.	Immediately dissolving; brilliant red.	Scarcely dissolving	Rapidly soluble; brilliant red.		
Ammonium hydroxid (concentrated).	Emulsified, dissolving, bright yellow.	Globules coalescing, slowly dissolving; orange, brown to yellow.	Rapidly soluble; yellow	Immediately soluble; orange to yellow.	Immediately soluble; orange to yellow.
Potassium hydroxid, 10 per cent.	Rapidly dissolving; bright yellow.	Globules rather rapidly dissolving; orange to yellow.	do	do	Do.
Potassium hydroxid, 10 per cent alcohol.	Immediately dissolving; bright yellow, becoming purple on heating.	Immediately dissolving; bright orange, becoming yellow on dilution.	Rapidly soluble; becoming purple on heating; yellow on standing.	More slowly soluble; orange to yellow.	More slowly soluble; orange to yellow.
Eau de Javelle	Swelled, emulsified, olive-green to yellowish.	Dissolving; yellow	Scarcely soluble		
Phloroglucin alcohol	Slowly dissolving; yellow	Slowly dissolving			
Phloroglucin alcohol plus hydrochloric acid.	No change	No change			
Vanillin plus hydrochloric acid.	Emulsified, dissolving; yellow; pink reaction appears slowly, at length very pronounced. Reaction appears in cortical tissues of root before it is seen in the glands.	Slowly dissolving; yellow, pink reaction in surrounding tissues; not in glands.			No reaction.
Alkannin	Darker, becoming reddened	Bright red, slow			
Sudan III in 70 per cent alcohol.	Dissolving without much color change.	Bright red, rapid; globules dissolving.			
Acid, osmic, 1 per cent	Secretion darkens, becoming black.	Blackened			
Potassium dichromate, 10 per cent.	Emulsified, brown particles not dissolving.	Partially precipitated; yellow to brown.			
Chromo-acetic fixative, 24 hours.	Solidified, not dissolving in alcohol; sulphuric red reaction fails or develops only slowly and partially.	Partially solidified; not dissolving in alcohol.			
Ferric chlorid, 10 per cent alcoholic.	Darkened; not dissolving	Dark green precipitate			
Ferrous sulphate, 10 per cent	Darkened; greenish	No color change			
Lead acetate	Precipitation from solution without much color change.	Precipitation without much color change.		Precipitated from solution; yellow.	Precipitated from solution; orange to yellow.
Cuprammonia	Darkened, emulsified, enveloping cells somewhat dissolving.	Scarcely dissolving; darkened, enveloping cells dissolved.			
Chlorzinciodin	Slowly emulsified; no cellulose reaction in enveloping cells of seed.	Cellulose reaction in enveloping layer.	Insoluble		
Iodin plus sulphuric acid		do			

Ammonium molybdate	Not much change of color in alkaline solution, with or without ammonium chlorid.	Orange-red in alkaline solution.	Not orange-red in alkaline solution.	.....	Orange-red in alkaline solution.
Copper tartrate in sodium hydroxid, warmed.	Reaction lacking or doubtful	Precipitate of copper oxid in varying degrees.	.....	.....	
Alpha-napthol in sulphuric acid.	Dissolving; bright green	Purple	Soluble; bright green	Soluble, bright yellow; becoming greenish in contact with air.	

Some discrepancies, especially in the matter of solubility, may readily be noted between the gland secretions and the pure substances which, the writers believe, make up their principal constituents. Two explanations, either or both of which may be correct, may be cited in respect to this point:

(1) Quercimeritrin and gossypol may be present in the form of salts whose solubilities differ from those of the pure substances. Perkin (*10*) believes quercimeritrin to exist in the petals of *G. herbaceum* in the form of a potassium salt. In proof of this view he cites (a) the ready solubility in water of the crude dyestuff from the corollas, in contrast to the relative insolubility of quercimeritrin; (b) the presence of a large quantity of potassium in the ash of his crude water extract; (c) the preparation from quercimeritrin of a monopotassium salt readily soluble in water. With respect to *G. hirsutum*, however, the writers do not find the gland secretion to be appreciably soluble in water (though this may be due to the protective action of an oil in which the flavone is dissolved. This will be again referred to). Also, as previously described (*17*) pure quercimeritrin has been prepared in this bureau directly by crystallization from an alcohol extract of corollas of *G. hirsutum*, without the treatment with lead acetate which Perkin employed with the species he investigated.

(2) The discrepancies in question may be due to the presence in the gland of other substances, especially of an ethereal oil in which the dyestuffs are dissolved. This oil would protect the dyestuffs against the action of reagents immiscible with it in which the solutes are less soluble (aqueous reagents). Conversely, the oil would render the dyestuffs apparently more soluble in reagents which mix readily with it—for example, ether—yet in which pure substances (quercimeritrin in the case of ether) are almost or quite insoluble. While no microchemical reaction has been developed which will definitely distinguish between quercetin and its glucosids, previous work (*17*) showed the presence in the corollas of a comparatively large amount of quercemeritrin, while the foliage yielded much quercetin and little quercimeritrin. It seems probable, therefore, that the glands of the corolla contain principally quercetin. This latter conjecture is strengthened by the sugar reaction given by the glands of the green parts, which indicates a probable enzymic hydrolysis.

SECRETIONS MORE OR LESS COMMON TO BOTH TYPES OF INTERNAL GLANDS

The presence of oil as a solvent of the flavone substances is indicated by the appearance of the globules and their ready coloration with alkannin, Sudan III, and osmic acid confirms this conjecture. The globules are reduced to solid form by treatment with dry heat at 100° C. or by steam; this and their ready solubility in alcohol indicate that the oil is of a volatile nature. A volatile oil whose properties are now under investigation has been prepared by the steam distillation of fresh cotton plants.

Other substances doubtless occur within the internal glands. A resin reaction is obtained by prolonged treatment with copper acetate. Prolonged treatment with chromic-acid reagents results in the conversion of apparently the whole content of the glands not exposed to light into a resinous-brown solid which is insoluble in water and alcohol and which gives the sulphuric-acid red reaction slowly, or not at all. The contents of those exposed to light form a yellow or dark-brown precipitate upon the walls of the cavity. This property of precipitation with chromic acid indicates that some of the contents of the glands may be closely allied to tannin.

Vanillin-hydrochloric acid gives, after several minutes, a strong phloroglucin reaction within the glands of the seed and root. None has been noted within the glands of the other parts, but phloroglucin is present within the adjoining cells. Sugar is frequently present in the glands exposed to light. Positive tests are given with α-napthol, sulphuric acid, and alkaline copper tartrate. The presence of sugar is somewhat doubtful in the other glands.

PRESENCE OF FLAVONE SUBSTANCES OUTSIDE GLANDS

In the green parts flavones are found only in the gland secretion. In the roots they occur in the outside cortical layers. They give the gossypol-red reaction and also a strong precipitation with tannin reagents. Pollen grains are colored bright red by sulphuric acid; those of *Malva silvestris* L. show a similar reaction. Flavones which agree with those of the green parts in forming a yellow solution with sulphuric acid occur in the seed, largely in the palisade layers of the cotyledons. Similar substances give the yellow coloration to the petals of *G. barbadense*, and are found to a less extent in the unfolding, nearly colorless or yellow corollas of *G. hirsutum*, being later obscured by anthocyans; but they are still capable of being shown by reagents. It is probable that the flavones in the glands form but a small proportion of the total amount present in the corolla.

RELATIONSHIP BETWEEN GOSSYPOL AND QUERCETIN AND ITS GLUCOSIDS

Marchlewski (7) terms gossypol a "dihydroxy phenolic substance," and proposes for it the formula $C_{13}H_{14}O_4$, with $C_{32}H_{34}O_{10}$ as an alternate formula. Withers and Carruth (*19*) consider that Marchlewski's substance contained acetic acid in combination. Their paper, which discusses the physiological effect of gossypol, does not deal extensively with the chemistry of the substance. They are now preparing publications which, it is to be hoped, will clear up the uncertainty which at present exists regarding the composition of gossypol. In the present state of our knowledge the exact chemical relation of gossypol to quercetin and its glucosids is uncertain. Perkin (*9*), referring to the work of Marchlewski on gossypol, states:

> It [gossypol] . . . does not appear to be closely allied with that (dye) present in the flowers.

This was before his discovery of quercimeritrin. Gossypol is evidently a somewhat unstable substance; Withers and Carruth (*19*) state that it readily forms an oxidation product which is physiologically inert. Its nature is also evidently changed by moist heat. Osborne and Mendel (*8*) find boiled cottonseed kernels to be nontoxic. The investigations of the present writers show that it is not formed, or if formed as a preliminary step in the synthesis of the quercetin glucosids, is immediately changed in those tissues which are exposed to the action of normal daylight. As gossypol occurs in the glands of the flower before opening and quercimeritrin after opening, the former apparently in this case gives rise to the latter; but the exact nature of the change remains uncertain. This change of gossypol differs from that in the unfolding of the cotyledons. Here no quercimeritrin seems to be produced, the gland content giving a greenish-brown emulsion with sulphuric acid.

RELATIONSHIP BETWEEN THE FLAVONES AND THE ANTHOCYANS

A definite relationship between the flavone glucosids and the anthocyans is assumed by Perkin (*11*) in citing the experiments of Everest (*3*), who formed anthocyans by a reduction of certain glucosids. The constant association of the two in the glands exposed to light, their immediate formation in those of the corolla as the petals unfold, and in the full-blown flower, the development of anthocyans in cells previously containing flavones and still containing them, point to a photochemical process resulting in the development of anthocyans from the glucosid. It is not asserted, however, that the development of anthocyan is here necessarily dependent on the preformation of glucosid in demonstrable quantities; anthocyan is frequently developed in the epidermal cells of stems, petioles, and leaves under extreme insolation where no flavone can be demonstrated. The stimulus of light, to which the development of anthocyan in normal tissue is here conjecturally referred, seems not to be invariably necessary. In the interior of certain bolls what appeared to be an abortive second boll developed, and in the outer tissues of this were developed glands analogous to those of normal tissue, containing no gossypol, but with pronounced anthocyan-bearing envelopes, although the stimulus of light was insufficient for the development of chlorophyll in the surrounding tissues.

POSSIBLE BIOLOGICAL SIGNIFICANCE OF THE INTERNAL GLANDS

Whether or not the substances found in the glands are of definite use to the plant is an unsettled question. Many investigators, notably Haberlandt (*4 p. 526*) have regarded the contents of glands of this general type as excretion products useless to the plant, basing this conclusion largely on the fact that they remain thus localized indefinitely without change. The change of gossypol upon the unfolding of the cotyledon may indicate its usefulnesss in the metabolism of the young seedling.

Furthermore, its pronounced toxicity may perhaps be regarded as a protective adaptation of the seed against animal attack. Haberlandt (*4*) states that—

> excretory substances (in reservoirs of this type) are frequently made use of for protection against animal foes.

Withers and Carruth (*19*) found rabbits much averse to cottonseed kernels as a food, especially after once having been made sick. The present writers have found no suggestion of such toxicity in quercetin or its glucosids, but have had no opportunity to test them biologically. Cook (*1*) credits the gland secretion with a repellent effect on the boll worm, and discusses its possible value in repelling the boll weevil. He finds that the relatively immune Kekchi cotton is usually punctured only in areas free from glands, but states also that glands are especially well developed in Mit Afifi and Egyptian cotton varieties particularly favored by the weevil. Boll weevils which have been watched while puncturing young plants seem to avoid the glands.

Preliminary experiments were made to test the attraction for the weevil of substances derived from the cotton plant. Of these the glucosids quercimeritrin, isoquerceretin, and especially the steam distillate and a volatile oil extracted therefrom appeared somewhat attractive. These substances are largely localized in the interior glands. It is possible, in view of the fact that the boll weevil has not been seen to puncture the glands, that this attraction may consist in an odor which suggests the presence of the cotton plant rather than in any actual food value of the substances. The flavone substances possess no odor perceptible to human senses; the volatile oil, on the contrary, has a pronounced and characteristic odor.

That the secretion of the glands is not repellent to all insects is shown by the habit of certain aphids (*Aphis gossypii* Glov.) in frequently puncturing the glands of the mature leaves and in withdrawing part of their substance (Pl. 49, A). The flavones present seem not to be appreciably diminished, and it is probable that the substance withdrawn by the aphids consists largely of sugar (dextrose), traces of which can be microchemically shown. This sugar may very likely be formed by the hydrolysis of glucosids. The indifference of the boll weevil to sweet substances has been previously noted by Hunter and Hinds (*6*).

UNIVERSAL PRESENCE OF INTERNAL GLANDS WITHIN THE GENUS GOSSYPIUM

Watt (*18*) makes the following statement:

> They [the glands] are, moreover, nearly universally present, though in some species they are often obscured by the tomentum.

He also refers to them in those of his specific descriptions which enter into such comparatively minor details. A considerable number of species of Gossypium in the National and Economic Herbariums have been exam-

ined, and in no case was there any notable variation in the presence and distribution of "black glands," although there was a great difference (often as great within species as between them) in their size and prominence. None of these specimens possessed roots. The names of the species examined follow:

Gossypium arboreum L.; *G. barbadense* L.; *G. brasiliense* Macf.; *G. herbaceum* L; *G. hirsutum* L.; *G. mexicanum* Tod.; *G. nanking* Meyen; *G. microcarpum* Tod.; *G. obtusifolium wightiana* Watt; *G. palmeri* Watt; *G. peruvianum* Cav.; *G. religiosum* Roxb.; *G. schottii* Watt; *G. tomentosum* Nutt.; *G. neglectum* Tod.; *G. wightianum* Tod.: all from the Economic Herbarium, Bureau of Plant Industry, and classified according to Watt (*18*). *G. drynarioides* Seeman; *G. harknessii* Brandg.; *G. davidsonii* Kellogg: at the Natioanl Herbarium.

That the chemical nature of the contents of the glands of these various species is identical by no means follows; Perkin (*9*, *11*) has demonstrated marked distinctions in the flavone content of the flowers of *G. neglectum*, *G. arboreum*, and *G. sanguineum*.

PRESENCE OF INTERNAL GLANDS IN GENERA CLOSELY RELATED TO GOSSYPIUM

Internal glands, such as are here described, have been noted within the Malvaceae only in certain genera of the subfamily Hibisceae. Senra, Lagunaria, Hibiscus, Abelmoschus, Kosteletzkia, and Dicellostyles appear not to possess glands of this type. Thespesia, Cienfuegosia (Fugosia), Erioxylon, and Ingenhouzia (Thurberia) are all more or less glandulate. The arrangement of glands in Arizona wild cotton (*Ingenhouzia triloba* Moç, and Sesse; syn. *Thurberia thespesiodes* A. Gray) is identical with that of *Gossypium* spp., and the glands of the seed (Pl. 42, B) react like those of that genus. Only a fragmentary specimen, consisting of stem, leaf, and flower of *Erioxylon aridum* Rose and Standley, has been seen by the present writers; on this the gland arrangement was also like that in *Gossypium* spp.

Four specimens of *Thespesia* spp. (*T. lampas* D. and E.; *T. populnea* Soland; *T. macrophylla* Blume and *T. grandiflora* D. C.) showed similar glands, but their arrangement was much less definite, and their presence (in herbarium specimens) sometimes could only be demonstrated by reagents. In contradistinction to the description of Thespesia by Schumann (*13*).

Kelch nicht punktiert,

the calyx of the two first species showed well-defined though inconspicuous glands. Seeds of both were densely glandulate, and the glands gave the gossypol-red reaction. Cienfuegosia is differentiated by Schumann (*13*) in part thus:

Kelch schwarz punktiert * * * Kotyledonen nicht punktiert.

Seven specimens were available for examination; only two specimens, *C.* (*Fugosia*) *drummondii* Lewtonand *C. phlomidifolia* Garcke, possessed

seeds, and those of the last named were nonglandulate. In *C. drummondii* very inconspicuous glands were present, distributed as in *Gossypium* spp.; on *C. phlomidifolia* (a poorly preserved specimen) they were not found on the leaves. The latter specimen only of those examined possessed glands on the petals. *C. australis* Schum., *C. hakeaefolia* Hochr., *C. argentina* Gürke, *C. hildebrandtii* Garcke, and *C. heterophylla* Garcke were also examined. In all but the second inconspicuous glands were present in most parts except the petals; usually they were most conspicuous on the involucre.

Dumont (*2*) cites the presence of "*poches schizogenes*" in species of Fugosia and Thespesia as in *Gossypium* spp.

II.—THE NECTARIES

Four sets of nectaries occur in the cotton plant, one set being floral and three extrafloral. The presence, shape, and number of the extrafloral nectaries vary in different species of Gossypium, and the taxonomic value of these variations has been discussed by Tyler (*16*).

The secretory mechanism of each nectary consists of a dense aggregation of pluriseptate glandular hairs, trichomes, or papillæ, among which simple nonsecretory hairs may be scattered. The development of the papillæ of the floral nectary from modified epidermal cells has been figured and described by Reed (*12*); and in accordance with the generally accepted origin of such structures, a similar development may be inferred for those of the two other sets. At certain periods these papillæ secrete a sweet fluid which attracts bees, moths, aphids, ants, and similar insects. Its saccharine nature is evident to the taste, and it yields the reaction characteristic of sugars with Molisch's, Meyer's, Flückiger's, and Fehling's reagents. There is no evidence to show that it is attractive in the least to the boll weevil, though its usefulness in attracting insects which prey upon this pest has been cited by Cook (*1*).

The comparatively long and narrow papillæ of the floral nectary line the inside of the base of the calyx in a band 1 or 2 mm. wide. In *G. hirsutum* and closely related species the nectary is guarded from the smaller insects by a band of hairs on the calyx above it. One set, usually consisting of three extrafloral nectaries within the bracteoles and alternating with them, occurs on the outer base of the calyx. These nectaries are irregularly triangular in shape and not deeply sunken. A set somewhat similar but more decidedly sagittate, is found deeply indented in the broadened apex of the peduncle, opposite the centers of the bracteoles, thus alternating with the intrainvolucral nectaries. Trelease (*14*), speaking of *G. hirsutum*, states that the nectaries of the outer calyx and peduncle are not present on the first flowers; on subsequent blooms those of the peduncle are formed, and on still later flowers both sets occur. In a variety of *G. barbadense* examined here the inner set appeared first, the outer set being absent on early flowers or represented by one nectary only.

A third form of extrafloral nectary (Pl. 50, A, B) is found on the underside of the principal foliar vein, not far from its base. These nectaries are shallow pits, varying considerably in shape and outline. On the cotyledon no pit occurs; the nectary is represented by a small group of poorly developed, nearly nonfunctional papillæ on the lower surface of the midvein (Pl. 49, B). On the leaves of *G. barbadense* each of the three principal veins usually possesses a nectary; that of the midvein is largest and usually sagittate in outline, with the point toward the apex of the leaf.

Internal glands are frequently found in the parenchymal tissues close to the nectaries (Pl. 49, B; 50, A, B), especially those of the leaf; but they have no organic connection with them.

Septate papillæ similar to those of the nectaries occur frequently on the young green parts of the plant.

SUMMARY

(1) Internal glands of lysigenous formation are found in the primary cortex, foliage, flower, and seed of Upland cotton (*Gossypium hirsutum*).

(2) The secondary cortex contains glands of a similar type. Some of these appear to be developed from the enlargement of a single cell.

(3) The glands in portions of the plant which are exposed to light are surrounded by an anthocyan-bearing envelope of flattened cells, and contain quercetin, probably partly or wholly in the form of its glucosids quercimeritrin or isoquercitrin, ethereal oil, resins, and perhaps tannins.

(4) The glands not normally exposed to the light are surrounded by a layer of flattened cells containing no anthocyans; they contain gossypol.

(5) Gossypol is formed in the glands of the developing corolla; on their exposure to light it is replaced by quercimeritrin.

(6) Gossypol in the unfolding cotyledons is changed, probably through oxidation, without the formation of quercimeritrin.

(7) Internal glands of the type described are universally present within *Gossypium* spp.

(8) Internal glands occur to some extent within the related genera, Thespesia, Cienfuegosia, Erioxylon, and Ingenhouzia.

(9) Four types of glands which function as nectaries occur in *G. hirsutum*. These differ morphologically from the internal glands and have no connection with them.

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PLATE 42

A.—Longitudinal section of a cotton seed, showing the internal glands in the cotyledons and the radicle. × 12.

B.—Longitudinal section of seed of *Ingenhouzia triloba*, showing the internal glands as in *Gossypium* spp. × 12.

C.—Internal gland of a cotton seed, with secretion. × 450.

A
B
C

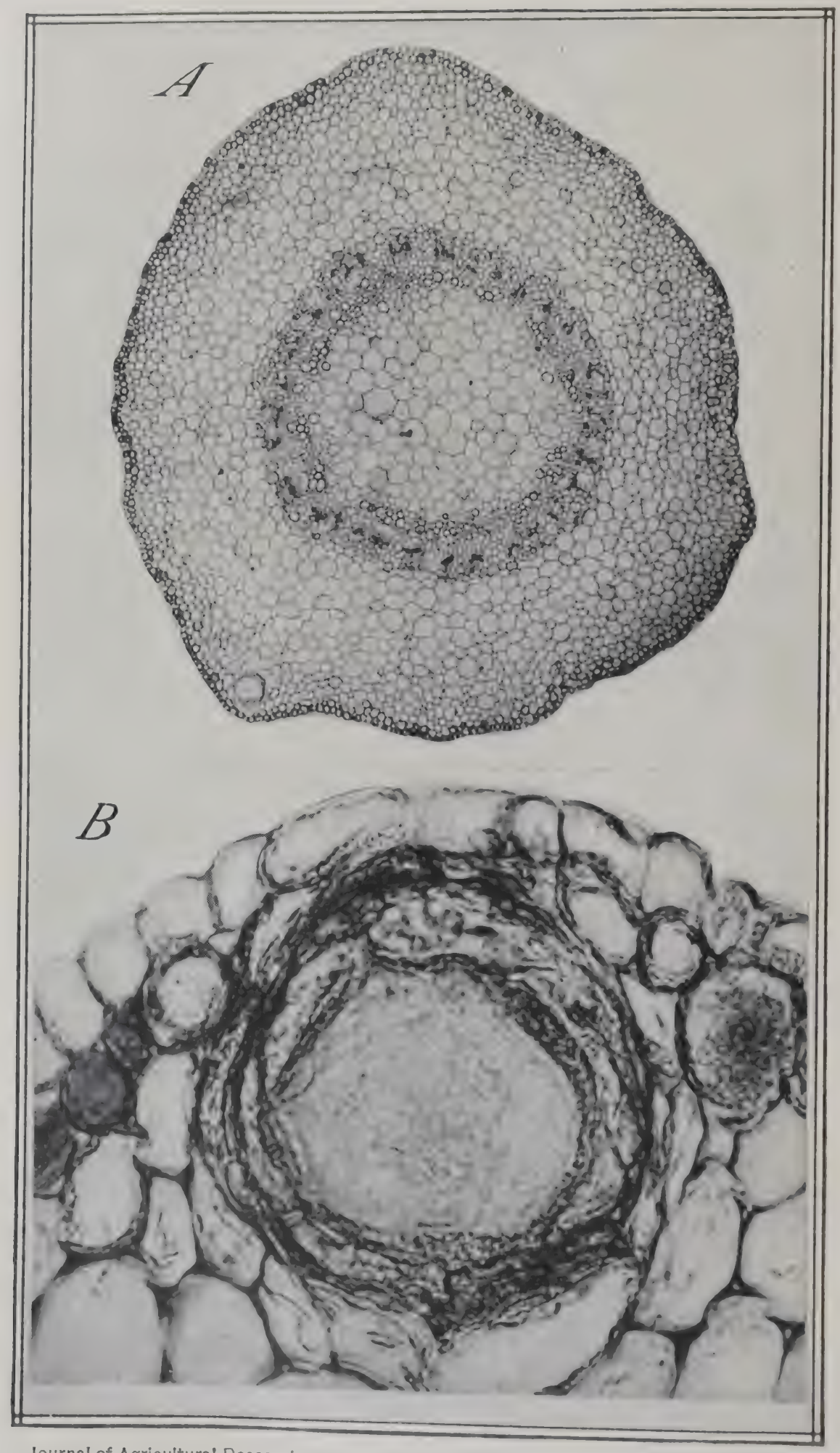
A
B

PLATE 43

A.—Cross-section of the hypocotyl of a cotton seedling, showing internal glands. × 48.

B.—Gland of same. × 655.

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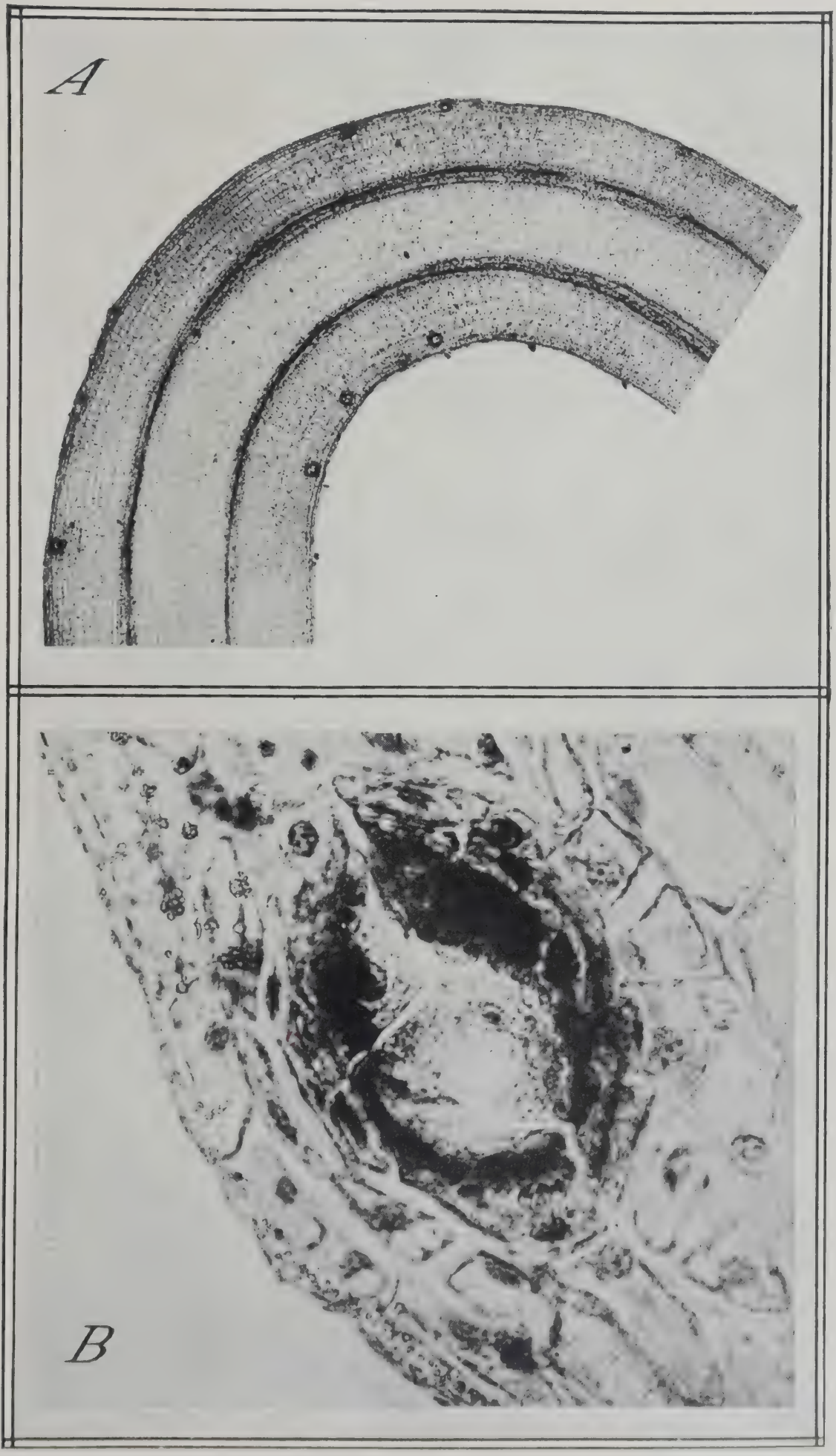
PLATE 44

A.—Longitudinal section of the hypocotyl of a cotton seedling, showing the internal glands. × 20.

B.—Gland of same. × 655.


A
B

A
B

PLATE 45

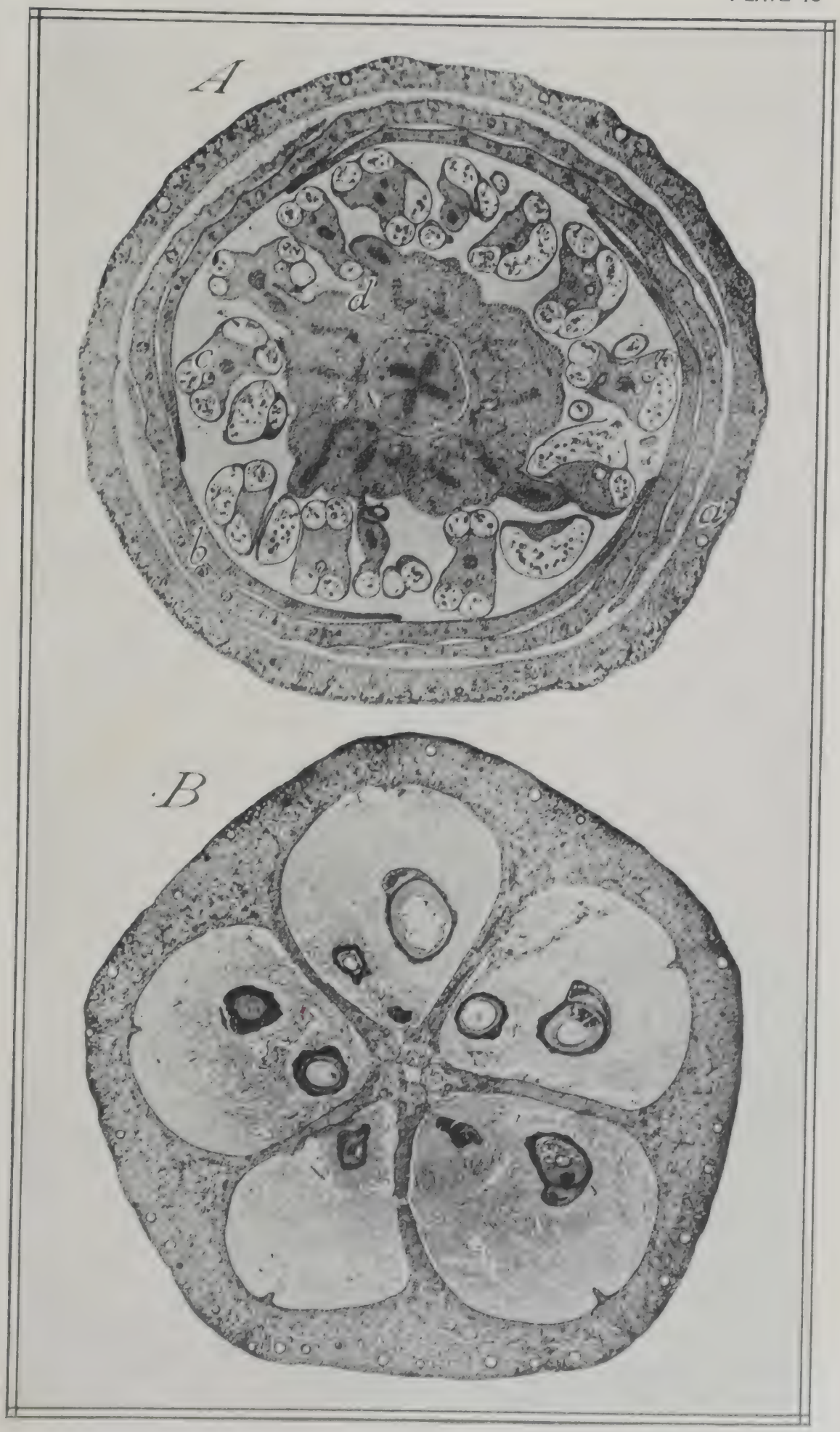
A.—Cross-section of a primary root of a cotton seedling, showing internal glands. × 38.

B.—Gland of same, the secretion having been removed by alcohol. × 585.

PLATE 46

A.—Cross-section of a cotton bud, showing internal glands in (*a*) calyx, (*b*) petal, (*c*) anther, (*d*) staminal column. × 15.

B.—Cross-section of a young cotton boll, showing internal glands. × 6.


A
d
c
b
a
B

A

B

PLATE 47

A.—Cross-section of a woody cotton stem, showing internal glands in the primary cortex (X), but none in the secondary cortex. × 11½.

B.—Cross-section of a phloem ray of a cotton root, showing two internal glands × 98.

PLATE 48

A–C.—Cross-sections of the internal gland of cotton from the ovary in the bud, showing three stages of its development. × 655.

A
B
C

A

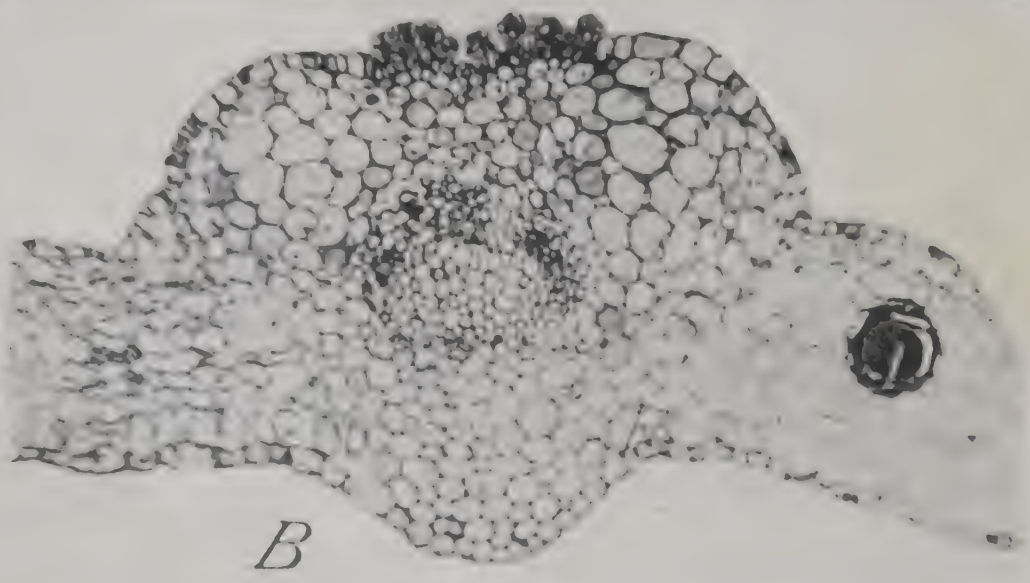

B

PLATE 49

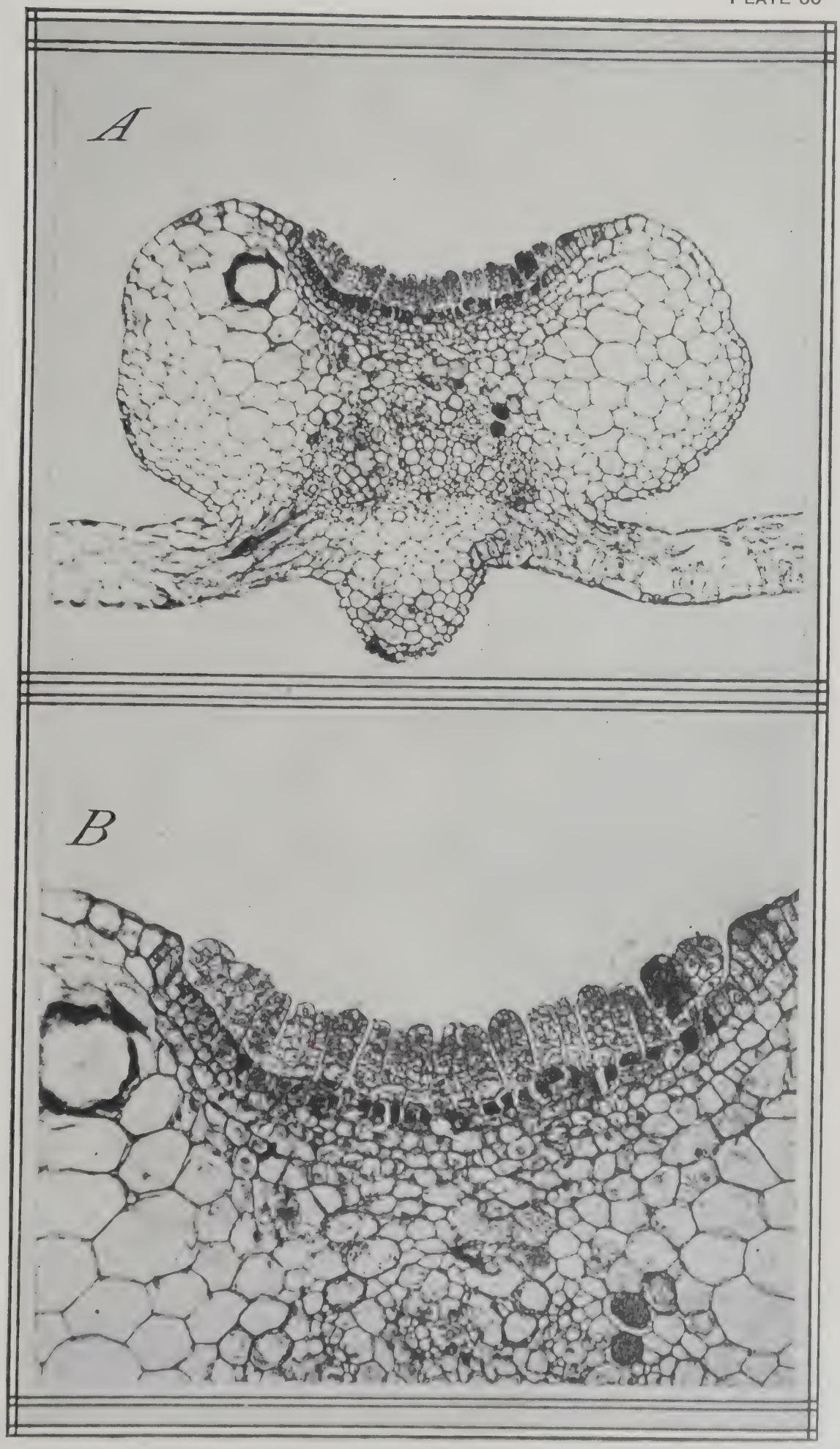
A.—Portion of a cotton leaf, showing internal glands punctured by aphids (surrounded by light area); also uninjured glands. Photographed by transmitted light. × 7.

B.—Cross-section of the midvein of a cotton cotyledon, showing rudimentary nectary. × 83.

PLATE 50

A.—Cross-section of the midvein of young true leaf of a cotton seedling, showing the nectary and internal gland. × 83.

B.—Nectary and internal gland of same. × 180.


A
B

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POX, OR PIT (SOIL ROT), OF THE SWEET POTATO

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INTRODUCTION

Next to the blackrot [cause: *Sphaeronema fimbriatum* (E. and H.) Sacc.] and stemrot (causes: *Fusarium batatatis* Wollenw. and *F. hyperoxysporum* Wollenw.), pox, or pit, may be considered as the most important and serious disease of the sweet potato (*Ipomoea batatas*). Until recently the trouble had been misunderstood, having been frequently mistaken for blackrot. For this reason the exact distribution of this disease and the money losses caused by it are not definitely known.

HISTORICAL REVIEW

Pox, or pit, of the sweet potato was first described in 1890 by Halsted (*5*) [1] under the name "soilrot," and was attributed by him to a fungus of a new genus and species named "*Acrocystis batatas* E. and H." Halsted's work was unchallenged for nearly 23 years. In 1913 the writer began the investigation of sweet-potato diseases at the Delaware Experiment Station, first studying the pox of the sweet potato. During the course of the investigation it became apparent that the fungus *Acroscystis batatas* was not the cause of pox, and it further became apparent that in reality the genus Acrocystis was nonexistent and that *A. batatas* was mistaken for another organism. In 1914 the writer (*13*) was the first to call attention to these facts in print. In the summers of 1914 and 1915 much time was spent in an infected field at Felton, Delaware, and more than 1,000 plate cultures were made of the various stages of the disease. At no time did the fungus *A. batatas* ever appear in culture. On the other hand, the predominant flora which constantly appeared were a species of Fusarium, an Actinomyces, bacteria, and a Rhizoctonia. Numerous inoculations of healthy sweet-potato roots in the field and in the greenhouse proved that the bacteria and the species of Fusarium were saprophytic in nature. The species of Rhizoctonia reproduced lesions that were very unlike the typical pox.

[1] Reference is made by number (italic) to "Literature cited," p. 449–450.

The Actinomyces was capable of producing a lesion the appearance of which resembled pox. In January, 1915, the writer severed his relationship with the Delaware Experiment Station, and assumed his present position. A project on sweet-potato diseases was at once undertaken at the Texas Agricultural Experiment Station. Pox was found to be as serious a disease in Texas as in Delaware. Greenhouse work was therefore undertaken as it was not difficult to obtain "sick" soil both from Texas and Delaware. Unfortunately, however, the field work was interrupted that season. At the Delaware Station Dr. J. A. Elliott continued the work of the writer on sweet-potato diseases. He (*3, 4*) verified the writer's previous conclusion that the fungus *A. batatas* was not the cause of pox. He further found that the disease was induced by a myxomycete of a new genus and species which he named "*Cystospora batata* Elliott." The object of the present paper is to verify Elliott's conclusions, and to add the observations and studies made by the writer.

NAME OF THE DISEASE

The term "soilrot" given by Halsted (*5*) is appropriate only in so far as it indicates that infection takes place on the underground portion of the plant. But it suggests practically nothing as to the nature or symptoms of the disease. In New Jersey the trouble is known to growers of sweet potatoes as "groundrot," a name more suggestive than "soilrot." In Delaware the nature of the disease was only vaguely understood; hence, it was variously known as "bugsting," "wormhole," "fertilizer-burn," and was often mistaken for blackrot. In Texas the disease has no definite name, but it is variously confused with the many root troubles of the sweet potato. In Virginia the disease is known to growers as "pox," or "pit," terms which best describe the trouble, and which were adopted first by the writer (*13*) and later by Elliott (*3, 4*).

GEOGRAPHIC DISTRIBUTION

There seems no doubt that pox has a wider geographical distribution than is at present known. It can probably be found wherever sweet potatoes grow. In New Jersey, Halsted (*5*) recorded it as a very serious, well-distributed disease. In Delaware it is as yet localized to Kent County, but appears to be gradually spreading southward. In Virginia the pox, although widespread, is at present localized in small areas. In Maryland the disease was recorded by Townsend (*14*), and was found by the writer to be a serious trouble, vying in importance with blackrot. In South Carolina pox was reported by Barre (*1*). In Texas it had been previously reported by Price (*11*); and from the writer's own observation, pox is a serious disease in this State. The same trouble seems also to be prevalent in Alabama, where it was recorded by Wilcox (*15*), and in Oklahoma as reported by Learn (*9*). The disease is also prevalent in Kansas, Prof. L. E. Melchers having recently sent specimens of it to the writer.

ECONOMIC IMPORTANCE

Pox is undoubtedly of great economic importance, but because of inadequate diagnosis estimates of the money losses from the trouble can not be given for all the States where sweet potatoes are grown. In a recent bulletin by Harter (*8*) there is no mention of this disease. This is certainly surprising when we consider the extent of the geographical distribution and economic importance of the disease. It may be safely stated that in fields where pox has become thoroughly established, the yields may be reduced by about 50 to 80 per cent. The writer has had occasion to make such estimates in many sweet-potato fields in Delaware, New Jersey, Maryland, Virginia, and Texas. These observations coincide with those of Halsted (*5*), who states—

> In some large fields visited this season [1890] the loss was almost total.

SYMPTOMS

In the literature pox, or soilrot, is poorly described, the symptoms of the disease not being fully given. The writer's extended field observations on the symptoms of pox may be summarized as follows: In badly affected fields the stand will be somewhat uneven. This, however, may not always be the case. That which attracts the attention most is thin growth, stunted vines, and a pale-green color of the foliage, all of which gives the impression of a very impoverished soil. In fact, growers do not attribute these conditions to the disease, but "to a lack of certain elements in the soil which past sweet-potato crops have removed." Such claims are unfounded, as these soils seem to produce good crops of corn, watermelons, etc. In pulling out a sweet-potato hill from a soil of this character one will be surprised to find an almost total lack of secondary feeding rootlets (Pl. 51, B). This is especially true when the examination is made at the season of maximum growth. Many of the feeding rootlets will be found totally destroyed, while others will exhibit numerous brownish spots at various intervals. Generally speaking, if infection starts at the tip of a growing root, the disease will work its way upward, and destroy that rootlet completely, leaving a discarded stub, which resembles the infected roots of other crops subject to the attacks of species of Thielavia. On the other hand, if infection takes place laterally, the resulting spot will be limited to about 0.1 inch. Frequently such roots may exhibit from 5 to 10 spots, each separated from the others by a healthy area (Pl. 51, A). The color of the spot is a deep chocolate-brown. Such infected rootlets, it is needless to say, become functionless. Besides attacking the feeding rootlets the pox also attacks the small roots which are destined to develop into edible roots (Pl. 51, A). This infection may be as severe and of the same character as in the feeding rootlets. Reduction in yield, lack of the normal green color, and limited vine growth may therefore be directly attributed to the destruction by the disease of the feeding rootlets and young roots.

Infection on the older growing roots may result in a constriction. Growth may seem to cease at this point, although it is uninterrupted on either side (Pl. 52). Infection on the older roots, besides misshaping them, does not, however, result in a total loss. Such roots usually attain a fair marketable size, and do not suffer in the least in edible quality. Here, however, the disease is manifested differently, from that on the young rootlets. On the older roots infection may be of two types.

The normal and typical one is characterized by small, dry, darkish, circular, more or less superficial spots the size of a dime or less. Later, the tissue of the spot in most cases dries up cracks (Pl. 52, A), and falls out, leaving a pox, or pit, whence the name of the disease. As a rule, a new skin is formed immediately below the area of the fallen spot. The depth of the spot seems to vary with the weather conditions; in dry weather the pox spots seem to enter more deeply into the tissue than during wet spells. In light cases of infection, there may be but one to three spots on the potato. In severe cases, however, the spots may be so numerous as to coalesce. These, on dropping out, leave a large, ragged, irregular pit. (Pl. 52, B). The tissue of the pox spot is dry and leathery, but is readily pulverized when rubbed between the fingers.

The second form of infection on the older potatoes is what Elliott (*4*) terms "blister" infection. It was observed by the writer but once; and in that case the infection took place at a feeding rootlet, then worked down to the main root, and was later apparent as a blister-like elevation on the epidermis.

CAUSE AND PATHOGENICITY

It has already been pointed out by the writer (*13*) that Halsted's fungus *Acrocystis batatis*, is not the cause of pox, although species of Rhizoctonia are frequently isolated from pox spots. The fungus, after repeated inoculation, failed to produce the typical disease. It is to the credit of Elliott (*4*) to have proved that pox is caused by a myxomycete *Cystospora batata* Elliott.

The pathogenicity of the organism may be readily proved in the following manner: Mature roots with unbroken pox spots may be easily secured. The roots are then carefully washed in running tap water to remove all trace of soil particles. They are then placed for two minutes in a mercuric-chlorid-alcohol solution,[1] and rinsed several times in sterilized water to remove all trace of the bichlorid. The roots are then placed in the dark in a sterilized moist chamber, heavily lined with clean, wet filter paper. In about 48 hours or more, a dark slimy mass will emerge from the now split pox spot. Upon transferring this slime to healthy sweet potatoes, which have been carefully disinfected and kept in a moist chamber, the typical disease may be reproduced in about three to five days.

[1] Made up of equal parts of mercuric chlorid (1:1,000) and 50 per cent alcohol.

Infection may also be obtained by placing a healthy root whose surface had been sterilized and then placed in contact with the slime developed from a pox spot in the moist chamber, as described above. The ease with which the slime organism may be produced from infected potatoes in the moist chamber should make it excellent material for class work. There seems no doubt that the slime mold *Cystospora batata* is the cause of pox. To prove this definitely, the writer has carried out the following experiment: The slime mold was produced aseptically from young pox spots, as previously described. Bits of the slime were transferred into the tubes of melted and properly cooled agar-agar, and poured into sterile plate dishes. The plates proved sterile, showing that there were no associated organisms in the slime which might be suspected of being the cause of pox. *Cystospara batata* refused to grow on petri plates in hard agar. This explains why the writer had failed in previous attempts to locate the causal organism of pox. In the larger number of plate cultures made, the organism never appeared from the infected tissue. This delayed the progress of the work. *C. batata*, however, may be grown artificially by the following method, suggested by Dr. T. F. Manns, [1] of the Delaware Experiment Station:

> The plasmodium may be grown upon a rich medium of ground up sweet potato, in which about five grams of agar is added per one thousand c. c. Use about 500 grams of such ground up sweet potato in a thousand c. c. of water, leaving as much food as possible in the medium, when made up to 1,000 c. c.

By taking out asceptically bits of tissue from young pox spots, and placing them in a flask in the above medium (sterilized in the autoclave at 15 pounds' pressure for 12 minutes), the organism will make fair growth. To establish further and more definitely the pathogenicity of *Cystospara batata*, six 7-inch pots were filled with typical sweet-potato soil, and sterilized in the autoclave for 12 hours at 20 pounds' pressure. After cooling down, four of these pots were inoculated on June 1, 1917, with the slime grown artificially on the sweet-potato medium in the flask, and the remaining two were left as controls. The inoculum was poured into the four pots and thoroughly mixed in the soil with a sterile spatula. All of the six pots were planted with healthy sweet-potato sprouts, two of the latter to each pot. The pots were kept in the laboratory where there was no possible chance for contamination. After three months all the pots were emptied and the roots in each carefully examined. The roots in the four pots which had been artificially inoculated with the slime grown in flasks showed definite symptoms of pox, whereas the roots in the two control pots were all healthy. This seemed to prove beyond any reasonable doubt that *C. batata* is the cause of pox of the sweet potato. Negative results were always obtained when bits of tissue taken from a pox spot where inoculated into a healthy sweet potato,

[1] Correspondence dated October 26, 1916.

and would seem to indicate that the active plasmodium is necessary for infection. This had already been shown by Elliott (4), who, however, also had claimed that infection of the growing tips of the tender rootlets takes place by means of amebæ.

METHODS OF SPREAD

It has already been stated that in dry weather the pox spot seems to be deeper. It is the general opinion of Halsted (5), Townsend (14), and Duggar (2), that pox is worse during dry weather. This in reality is true only in so far as the pox spots are deeper, and cause much more visible damage by distortion and disfigurement of the marketable roots. In wet weather pox is just as severe, and the causal organism perhaps more active, but the spots are more shallow and less noticeable. The roots are not so disfigured, and, hence, are more saleable. According to Halsted (5) and others, pox is spread about from field to field by wind-blown spores of the causal organism. The work of the writer does not seem to bear this out. Extended observations and studies have shown that pox does not spread readily from field to field nor even to adjoining neighboring fields by means of wind-blown spores. If it did, the disease would spread very rapidly over large areas. This, however, is not the case. Pox will not become very noticeable until 8 or 10 years after its introduction in a field, and then only when the crop is continually grown on the same land. The disease does not seem to spread rapidly from an infected field to the neighboring fields, but advances slowly, unnoticed and unsuspected. Definite evidence is also lacking as to whether pox is carried over on the small potatoes (seeds) in storage. As a rule, sweet-potato growers never hesitate to plant infected stored seed. According to the writer's observation, and numerous communications from growers, sprouts from such seed, when pulled for transplanting, have not shown evidences of pox on any of the underground portions of the plant. This evidence is further strengthened by Duggar (2), who states that—

> Soil rot has not been observed to spread by way of the hot-bed, but only through contamination of the soil of the field.

In order to verify this, the writer has often planted infected seed which wintered over in storage in a sterilized soil. These seeds were surface-sterilized in the usual way. At no time, however, did the resulting sprouts show any marks of pox. The mode of spread of pox needs further careful investigation. It seems reasonable to suppose, however, that the disease is probably disseminated with lumps of soil which have been carried on farm implements on wet days. At that time the sandy soil is more likely to stick together. It is also likely to spread with diseased sprouts which had been grown in a seed bed the soil of which has been taken from a previously infected field. Washing by rain is also likely to carry the disease in the field. In lands with a natural slope pox

will be seen to spread downward in the direction of the water-fall—that is, from the highest to the lowest point—but seldom in the opposite direction.

IS POX A STORAGE TROUBLE?

Elliott (*3*) states that—

> A secondary infection by swarm spores in small immature "pits," causing extensive, blister-like elevations in the skin of stored sweet potatoes, has been observed.

While there seems no doubt of the observation of these blister-like elevations, it seems very probable that the infection did not occur in storage, but late in the field at digging. When these roots were taken in storage, incubation probably was very slow, and because of unfavorable indoor temperature conditions, infection resulted, not in normal spots, but in blisters. In fact, the above supposition is evidently supported by Elliott (*4*) himself in the following statement:

> On infected roots kept in the laboratory, in a dry chamber, a hitherto undescribed secondary infection was observed [referring here to the blister infection].

It seems safe to state that pox is primarily a field trouble, and not a storage trouble. In fact, growers in the infected districts prefer not to sell the infected crop when freshly dug, but to store it over the winter. In the storage house the pox spots dry, and by the time the roots are ready to be shipped, most of them have fallen out. Similar observations are also recorded by Townsend (*14*) and Wilcox (*15*).

OTHER CROPS SUSCEPTIBLE TO POX

As will be seen presently, pox attacks not only the sweet potato but other hosts as well. In the summer of 1914 an old Virginia grower stated to the writer that he never plants white (Irish) potatoes (*Solanum tuberosum*) on the same land where sweet potatoes affected with pox have grown for "the white potato, too, is subject to the same disease." Upon further inquiry it was found that the same practice was observed by most growers there. Bearing this in mind, in 1915 the writer planted Irish potatoes side by side with sweet potatoes in a field badly infected with pox. Observations were made from time to time by pulling out growing plants. Unmistakable symptoms of the pox were noticed at a very early stage. At harvesting, about 60 per cent of the tubers were affected with pox. The symptoms on the Irish potato were no different from those of the pox on the mature roots of the sweet potato (Pl. 51, C-E). However, the spots on the Irish potato seemed to be more shallow. Potato growers in Virginia maintain that some varieties of Irish potatoes seem to be more resistant to pox than others. The Irish Cobbler seems to be the least resistant. This statement is worthy of further investigation. That pox is a serious disease of the Irish

potato there seems no doubt. Dr. T. F. Manns,[1] of the Delaware Station, writes as follows:

> The disease (pox) has proven very severe this year on white potatoes in lower Delaware and Maryland, some specimens being worse than any specimens of scab I believe I have ever seen.

Elliott (*3, 4*), too, has recorded the Irish potato as a host to pox. There seems no doubt that the disease on the Irish potato is far more widespread than has heretofore been recognized; it is very probable also that it has been mistaken and confused with other troubles. Morse and Shapovalov (*10*) and more recently Ramsey (*12*) in their work on the disease caused by *Rhizoctonia* sp., have noticed a pitting disease of the Irish potato that has been attributed to that fungus. No doubt *Rhizoctonia* sp. is abundant in these pits, but from the illustrations given by Morse and Shapovalov (*10*) it is evident that they were dealing with the pox, and that the trouble caused by species of Rhizoctonia was merely secondary.

The writer and Elliott (*4*) have already stated that the pox spots on the Irish potato are of a shallow type; however, under Maine conditions, it is very probable that *Rhizoctonia* sp. merely enters as a result of the injury caused by *Cystospora batata*, and that having once penetrated, it is capable of working in farther, thus deepening the pit. Prof. Ramsey was kind enough to send the writer slides of his so-called "Rhizoctonia pits." In every case these slides were sections of cracked "pits." A careful examination showed a few remaining cysts irregularly scattered in the tissue of the "pit" area. Furthermore, the largest quantity of filaments of *Rhizoctonia* sp. are found in the center, at each side of the crack of the "pit," a place from which the invading plasmodium migrates back to the soil. It seems very probable that the growth of *Rhizoctonia* sp. in the "pits" is limited by the secretion of a toxin which the plasmodium of *C. batata* leaves in the occupied cells before migrating.

Pox on the Irish potato has so far been found to attack the tubers only, and not the roots and rootlets, as it does with the sweet potato. Infection apparently takes place at a lenticel, as Ramsey (*12*) also found.

The turnip (*Brassica rapa*) is also susceptible to pox. In the summers of 1916 and 1917 the writer sowed turnip seed in soil infected with *Cystospora batata*, on which sweet potatoes had been badly diseased. A large percentage of the turnips showed unmistakable pox infection. Here, however, the spots were more superficial than on the Irish potato. It is now suspected that the beet (*Beta vulgaris*) and tomato (*Lycopersicon esculentum*) are also subject to the attack of this disease. Further studies on these two hosts are now in progress.

[1] Letter dated November 15, 1916.

MORPHOLOGY AND LIFE HISTORY OF THE ORGANISM

The gross morphology of *Cystospora batata* has already been indicated by Elliott (*4*). The organism must undoubtedly hibernate in the soil as cysts. This stage probably enables it to resist drouth and cold. Careful experiments by the writer have shown that freezing will not affect *C. batata* in infected soil. Pox-sick soil in flowerpots exposed to outdoor freezing weather during the entire winter will not show the least weakening in the virulency of the causal organism. Similarly, ordinary drying for 12 months will have no injurious effect on *C. batata* in the infected soil.

The cysts are heavy-walled, and each individual may contain large numbers of swarm spores or amebæ. When the latter are ready to emerge, the cyst wall becomes thinner, until finally the swarm spores break through. Infection of the host may take place by the penetration of individual amebæ into the epidermal host cells. This is especially the case with root tips. Ordinarily, however, infection is by means of a plasmodium or of both methods. The swarm spores are round, but slightly tapered at both ends, and possess a single, short flagellum. Occasionally the swarm spores fuse in pairs, but from the writer's observations this has not been the rule. They are usually active after emergence from the mother cyst. The period of activity, although varying from 1 to 7 days, is usually short, often less than 30 minutes. They gradually increase in size, taking on the ameboid, then the plasmodial form. At this stage a large number of nuclei are formed by mitotic division. Nuclear division seems to proceed by a definite mathematical ratio of 1, 2, 4, 8, etc. Single plasmodia may often contain from 200 to 300 nuclei. At this stage and before escaping, the plasmodium becomes more dense, and thickly granular in the center, surrounded by a clearer zone which later becomes a thick cell of the cyst. The latter apparently undergoes a short period of rest, during which time the swarm spores are formed. These in turn emerge and undergo the same life cycle as above described. Thus, in a single infected root tip or in a pox spot, several crops of swarm spores may be formed within the host cells, each generation of which advances farther. Finally all the plasmodia seem to collect, cease advancing, turn backwards, and leave the pit for the soil. It is probable that the plasmodia in the soil encyst and pass the winter in that way. Numerous cytological and morphological studies of this important organism are still in progress.

A NEW SPECIES OF ACTINOMYCES ASSOCIATED WITH POX

Of the many bacteria and fungi isolated from pox, a species of Actinomyces is very often obtained from diseased spots. Because of the persistence of this organism, work was undertaken on it by the writer. Inoculation experiments with pure cultures of this organism showed that

it is capable of producing a spot which, although not resembling pox, may penetrate equally deep in the host tissue. This species of Actinomyces was isolated from sweet-potato material in Delaware, as well as from specimens grown in Texas. Cultures of the organism were grown on various media, and parallel with a strain of *Actinomyces chromogenus* from the Irish potato secured through the kindness of Dr. W. J. Morse, of the Maine Experiment Station. The two organisms appeared to be distinct, and it was thought that the species of Actinomyces grown on sweet potato was a new one, to which the name "*Actinomyces poolensis*, n. sp." was given. Upon submitting a culture of this sweet potato organism to Dr. H. J. Conn, of the Geneva Experiment Station, it was also pronounced by him to be a new species. *A. poolensis* and *A. chromogenus* were grown parallel on numerous culture media in plates and on slants. The greatest differences are observed when the two organisms are grown on potato plugs, corn-meal agar, and on Cook's media No. 2.[1] The difference between the two organisms may be summarized as follows:

	Actinomyces poolensis.	*Antinomyces chromogenus.*
Media No. 2 slants	No color produced in media. Growth slow, thin, flat to undulated up to six weeks after which it becomes more abundant than in *A. chromogenus*. Creamy, glistening, becoming dirty-cream with age.	Media colored from brown to black. Growth rapid. Thick rays, ruffled white. After six weeks, growth slackens, surface becomes brownish.
Corn-meal-agar slants	Media not darkened. Growth abundant, flat, deeply ingrown in substratum. Yellow border with raised white center.	Media not darkened. Growth scant, ingrown in substrata but wavy. Color partially white to gray.
Potato plugs	Potato not colored. Growth slow, thin, wavy, light brown jelly-like, not glistening.	Potato blackened, growth slow, but abundant, spreading, raised, wavy, glistening.

A pure culture of *Actinomyces poolensis* was also submitted to Dr. S. A. Waksman, of the New Jersey Experiment Station, who reported as follows:

Your second culture, "Pox from sweet potato," has also been isolated by us from several soils. As far as I am aware, this organism has not been described as yet. In a series of biochemical investigations on Actinomyces this organism was found to possess strong proteolytic activities, which may perhaps serve as a clue to its pathogenicity. The cultural characters of this organism are as follows:

A very good growth is produced on different organic media and also on synthetic media containing glucose or glycerine. A good, but uncharacteristic growth was produced on Lubenau's egg medium, Petroff's medium, glycerine, beef infusion agar and Leoffler's blood serum. Growth restricted, cream colored, aerial mycelium gray to

[1] Water (distilled) 1,000 c. c., agar 15 gm., glucose 20 gm., peptone 10 gm., dipotassium phosphate 0.25 gm., magnesium sulphate 0.25 gm.

musty gray on Krainsky's Ca-Malate agar; thin cream colored growth, surface becoming with an ash-gray aerial mycelium, on Czapek's solution agar, in which glucose or glycerine took the place of sucrose, the growth is heavy, yellowish, aerial mycelium abundant, gray (glucose) or (white). No soluble pigment is produced on any of the media studied. On potato plug the growth is light brown, no aerial mycelium is produced, plug is not colored. Milk, at 37° C. is hydrolized in 15 days. Gelatine, at 15° C. is slowly liquified (10 mm. on 20 days), with no color production in the liquefied portion; the growth is light brown, with no aerial mycelium. On glucose broth a flocculent uncharacteristic growth is produced. The organism grows very readily on all the media at 37° C.

Microscopically the following points are to be noted: Spirals are not produced; the aerial mycelium soon breaks up into short cylindrical spores, although many spherical spores are found.

Actinomyces poolensis is a superficial wound parasite, usually found following the pox spots produced by *Cystospora batatas*. The former organism will not grow on healthy tubers of the Irish potato. Structurally *A. poolensis* and *A. chromogenus* differ very little. They can be distinguished only pathologically and when grown parallel on different media.

THE GENUS ACROCYSTIS NOT VALID

In describing pox (soilrot) Halsted (5) has figured a new fungus of a new genus and species which he named "*Acrocystis batatas* E. and Hals." The latter was practically the only described species of the genus Acrocystis. However, Halsted's drawings of Acrocystis are really mistaken figures for *Cystospora batata*, a myxomycete, and not a fungus. Elliott (4) and the writer have proved that pox of the sweet potato is caused by a myxomycete, *Cystospora batata* Elliott. It is therefore evident that *Acrocystis batatas* does not exist at all, and that the genus Acrocystis is not valid.

METHODS OF CONTROL

A careful search through the literature seems to show that Halsted (6, 7) was practically the first to carry out extensive field work on the control of pox. He found that time has a great tendency to increase the spread of pox and at the same time materially to decrease the yield. On the other hand, he discovered that a broadcast application of 300 or 400 pounds of both sulphur and kainit per acre would decrease the disease and also increase the yield of marketable potatoes. The experiments as carried out by Halsted (6, 7) are now being duplicated by the writer in the greenhouse, where conditions are more under control. The work, however, has not progressed far enough to justify any positive statements at this time. From a practical point of view the writer decided to ascertain whether an alkaline or an acid fertilizer would favor or control pox in the field. Accordingly an infested field that had been chosen received normal application of 1,000 pounds per acre of a potash phosphate with the following guaranteed analysis: Ammonia 6 to 8 per

cent, available acid phosphate 7 to 8 per cent, potash 5 per cent. The land was then divided into three plots. The middle remained as a control, and received no further treatment. The plot to the right received an additional application of acid phosphate (guaranteed analysis, 14 per cent of available phosphoric acid) at the rate of 2,000 pounds per acre. The plot to the left received an additional application of hydrated lime (guaranteed analysis, 65 per cent of calcium oxid) at the rate of 2,000 pounds to the acre. The results obtained were very striking. The control plot gave an average of almost 60 per cent affected roots. The lime plot increased the amount of affected roots to about 85 per cent. The percentage of diseased roots in the acid-phosphate plot was 32. This seemed to indicate that an acid fertilizer has a tendency to keep the pox in check, whereas lime has the opposite effect.

The work of the writer would also seem to indicate that there exist considerable differences in the resistance of varieties of sweet potatoes to the disease. Of the limited number tested in 1915 in Delaware the following is a tentative classification of their resistance: (a) Total freedom from disease (Dahomey, Red Brazil, Pearson); (b) from 1 to 20 per cent infected (Big Stem Jersey, White Yam, Yellow Strassberger); (c) from 20 to 90 per cent infected (Goldskin, Big Leaf Upriver).

Steaming the soil for six hours at 20 pounds' pressure will free it from the pox organism. This also seems to be true when the infected soil is treated with formaldehyde at the rate of 1 pint in 20 gallons of water, applied at the rate of 1 gallon of the solution to each square foot of soil space. However, since it is very doubtful if the disease is carried with the seed in the soil of the seed bed, soil sterilization would hardly seem warranted unless it aimed also at controlling blackrot. Likewise it seems hardly necessary to treat the seed for that alone. It is not definitely known how long *Cystospara batata* would persist in the soil without a suitable host. Observations of practical growers differ greatly in this respect. Some assert that at least a 10-year rotation is necessary to free the land from pox; others, and these seem to be in the majority, maintain that a 3-year rotation is sufficient. Soil conditions, it seems, play an important factor. Pox is more severe in the lightest of the sandy soils, and less so in the heavy clay loams.

SUMMARY

(3) Although not definitely known, pox is probably prevalent wherever sweet potatoes are grown. The name "soilrot" does not express the symptoms of the disease and the terms "pox" or "pit" are recommended.

(4) Pox disfigures the root and reduces yields. It destroys the feeding rootlets and many of the roots which otherwise would make the crop.

(5) Infections which result in blisters are apparently the exception, and not the rule, and may be brought about by conditions unfavorable for the parasite.

(6) Pox is caused by a myxomycete, *Cystospara batata* Elliott, and not by the fungus *Acrocystis batatas* E. and H.

(7) The disease may be reproduced at will artificially. Infection can not take place by inserting bits of diseased tissue into healthy parts. The contact of healthy roots with the active slime is necessary for artificial infection. The organism may be grown artificially on culture media.

(8) Pox seems to be equally active in wet and in dry weather. The greatest damage, however, occurs in dry weather, when the roots seem to be more distorted.

(9) In the field the disease does not seem to be disseminated by free-wind-blown spores (cysts) of the causal organism; nor does it seem to be carried over on infected roots kept in storage. It seems to spread with lumps of soil which may adhere to the working tools in wet weather. Evidences also tend to show that the disease is disseminated in the field by rain water.

(10) Pox seems to be a field trouble only.

(11) Pox also attacks the Irish potato and the turnip. The beet and tomato are suspected of being susceptible hosts. There seems little doubt that Morse and Ramsey, in attributing the "pit" disease of the Irish potato to *Rhizoctonia* spp., were in reality dealing with pox, the injury by *Rhizoctonia* spp. being merely secondary.

(12) *C. batata* probably hibernates as cysts in the soil.

(13) A new species of Actinomyces, *A. poolensis* Taubenhaus, is found associated with the *C. batata*. The former acts only as a wound parasite and secondary invader.

(14) The genus Acrocystis, as originally described by Halsted, is not valid and is nonexistent. The fungus *Acrocystis batatas* was mistaken by Halsted for a myxomycete which Elliott has named "*Cystospora batata*," the true cause of pox (soilrot).

(15) The red varieties of the sweet potato seem to possess the greatest resistance to pox.

(16) Soil sterilization of the soil of the seed bed to control pox is not recommended. Rotation of crops tends to decrease the disease in a sick soil. The rotation remains to be worked out.

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PLATE 51

A.—Young sweet-potato roots affected with pox spots.

B.—Sweet-potato sprouts, the lower rootlets of which have been totally destroyed by pox.

C.—Typical pox spots on tubers of the Irish potato.

D.—Pox spots of the Irish potato (after Ramsey).

E.—Pox on Irish potato showing lenticel infection.

A
B
C
D
E

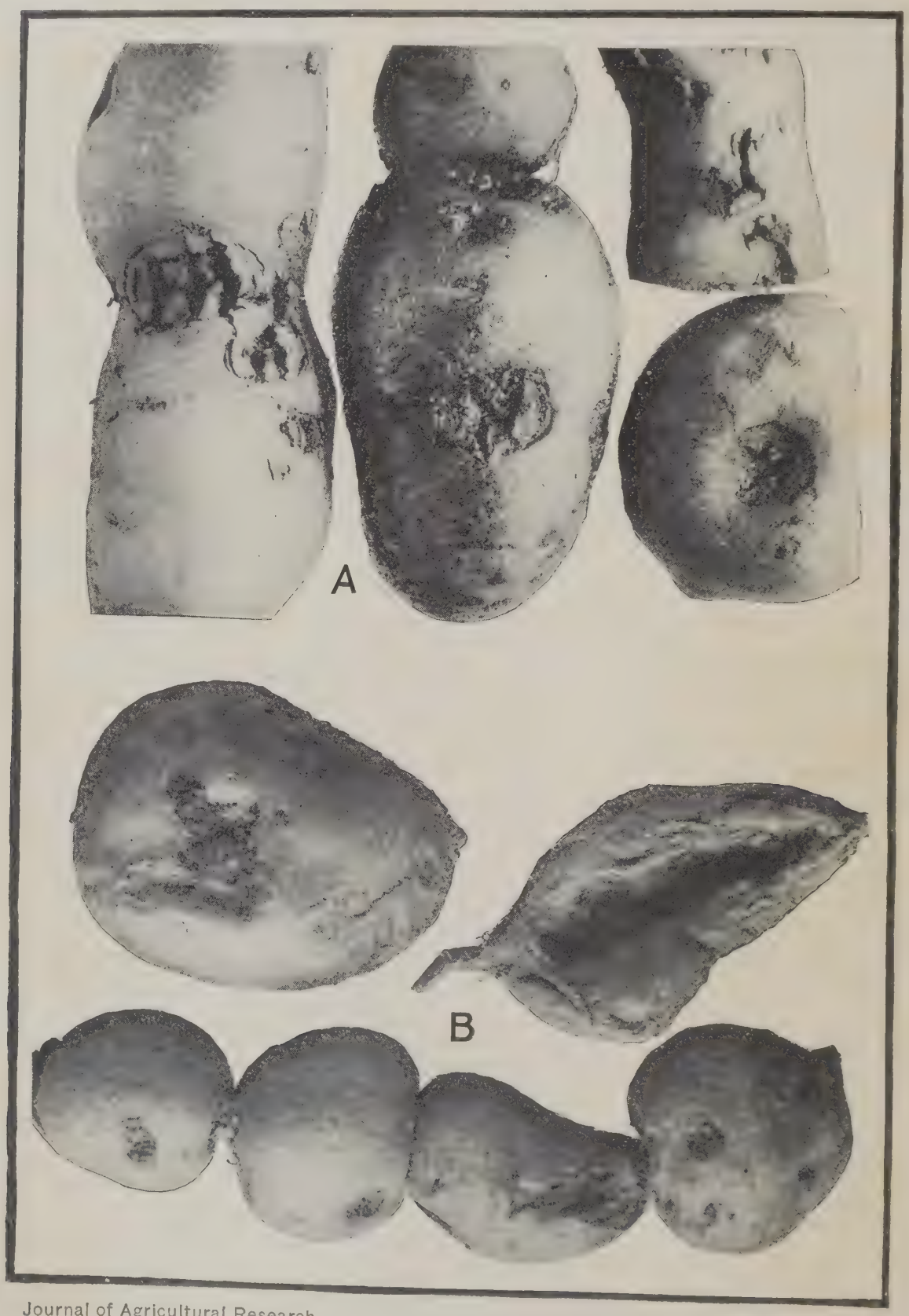
A
B

PLATE 52

A.—Sweet potatoes showing the typical pox spots and cracking previous to the falling out of affected tissue.

B.—Top row: Sweet potatoes showing the large pits formed as a result of a heavy infection, and later by the falling out of the pox spots. Bottom row: Sweet potatoes showing the constricted effect and uneven growth of the root as a result of early infection.

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BORON: ITS EFFECT ON CROPS AND ITS DISTRIBUTION IN PLANTS AND SOIL IN DIFFERENT PARTS OF THE UNITED STATES

By F. C. Cook and J. B. Wilson, *of the Animal Physiological Chemical Laboratory, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

The results reported in this paper are a continuation of the experiments previously recorded (*2*, *3*, *4*),[1] dealing with the effect on the growth of crops of borax and colemanite (calcium borate) when added to horse manure in amounts sufficient to kill the larvæ of the house fly, with particular reference to the action of boron-treated manure when applied to the same soil for two or three consecutive seasons. It was thought that crops that had not been injured by the first addition of the boron-treated manure might be injured by a second or third application. A study was made of the effect on crops and soil of large amounts of boron-treated manure such as might be used by truck growers to force crops. In this way the tests covered the application of the largest possibly amounts of boron-treated manure to soil which might possibly be made through any combination of circumstances.

GENERAL PLAN OF EXPERIMENTS[2]

In all tests 0.08 pound of borax or 0.095 pound of colemanite per bushel of manure were used. These quanities are sufficient to act as a larvicide for the house fly. When 0.08 pound of borax is added to the bushel of manure and applied at the rate of 16 tons to the acre, it is calculated, by assuming that the weight of the upper 6 inches of soil per acre is 1,750,000 pounds, that 0.00176 per cent of boron as boric acid (H_3BO_3) is present in the upper 6 inches of the soil. When the above quantity of colemanite is added to the manure, 0.00232 per cent of boric acid is estimated to be in the upper 6 inches of soil. If the treated manure is applied at the rate of 24 and 40 tons to the acre, it is calculated that 0.00264 and 0.0044 per cent, respectively, of boric acid are present in the upper 6 inches of soil when borax is added. When colemanite is added, 0.00348 and 0.0058 per cent, respectively, of boric acid are present in the same amount of soil.

The action of both borax-treated manure and colemanite-treated manure on barley and peach trees in California and on wheat at Benton

[1] Reference is made by number (italic) to "Literature cited," p. 470.

[2] The experiments were carried out with the cooperation of Mr. W. D. Hunter, of the Bureau of Entomology.

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Harbor, Mich., was tested during the season of 1914–15. In these experiments but one application of the boron-treated manure was made, and the observations extended over only one season. The manure was applied to the soil at the rate of 16 tons per acre. The amount of boric acid calculated to have been added to the soil is given above.

Experiments were inaugurated in 1914 on the experimental farm of the Bureau of Plant Industry at Arlington, Va., and on the farm of the Bureau of Animal Industry at Bethesda, Md. The Arlington experiments extended over two seasons; those at Bethesda over three seasons. The manure was applied to the soil at the rates of 16, 24, and 40 tons per acre. (The percentage of boric acid in the different plots is given on p. 451.) Nine plots were used at each place: Three for borax-treated manure, three for colemanite-treated manure, and three for untreated manure. In all cases the boron was well incorporated with the manure, and the mixture then stood in piles for 10 days before it was spread on the plots. The ground was lightly plowed, harrowed, and rolled. Different vegetables were grown on the plots the first two seasons. Rye was grown the third season on all plots at Bethesda.[1]

In order to obtain additional information in regard to the length of time that boron can be detected in soils, samples of soils which had received applications of boron-treated manure in 1914 and which had been tested for soluble boron in 1915 were taken in 1916 from the plots at Orlando, Fla., New Orleans, La., and Dallas, Tex.

EXPERIMENTAL METHODS USED

Boron was estimated in the plants and soil as boric acid, with the methods described by one of the writers (*2*). This procedure involved the use of strips of curcumin paper, and a comparison of the color obtained from extracts of the ash of the plants and fruit and extracts and fusion mixtures from the soil, with the color from standard solutions of boric acid. Both total and acid-soluble boron were estimated in the soil.

The methods of the Association of Official Agricultural Chemists [2] for solids, total nitrogen, and for nitrogen as ammonia on distillation with magnesium oxid were used. The Folin and McCallum aeration method for ammonia [3] and the aluminum-foil reduction method for nitrates [4] as adopted by the American Public Health Association were employed.

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[1] The cooperation of Dr. E. C. Schroeder, of Bethesda, and of Messrs. Butterfield and Criswell, of the Arlington Farm, was of great assistance.

[2] Association of Official Agricultural Chemists. REPORT OF THE COMMITTEE ON EDITING TENTATIVE AND OFFICIAL METHODS OF ANALYSIS. 381 p., 15 fig. Baltimore, 1916.

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EFFECT OF BORAX AND COLEMANITE ON PEACH TREES

Boron-treated and colemanite-treated manures were applied to the soil of separate groups of peach trees (*Amygdalus persica*) in an orchard at Acampo, Cal., in November, 1914. A third group of trees was fertilized with untreated manure, and a fourth group was left unfertilized. A total of 100 trees were included in the experiment. The work was done under the supervision of Mr. R. L. Nougaret, of the Bureau of Entomology. The orchard was planted in the spring of 1913. The manure was applied at the rate of 16 tons per acre. The amounts of boron and colemanite added to the manure are given on page 451. In the fall of 1915 the groups of fertilized trees could be easily distinguished from the other trees in the orchard because of the vigorous growth and dark-green color of the foliage. The dark-green foliage was particularly marked in the case of the trees fertilized with borax-treated manure, the foliage of the trees fertilized with colemanite manure being somewhat less green, while the foliage of the unmanured trees was the least green. Three nurserymen having no knowledge of the nature of the experiment passed judgment on the length of the growth of new wood, the fruit buds, and the general condition of the trees. All of the trees treated with either borax-treated or colemanite-treated manure were given the highest ratings, while many of the trees fertilized with untreated manure or left unfertilized were given lower ratings.

Samples of soil from the experimental section of the orchard were tested for boron. No acid-soluble boron was found in any of the soils, but there was a small amount of total boron in all of the soils. The results are recorded in Table I and are in agreement with the results of the analyses of other soils in showing the absence of detectable amounts of soluble boron.

TABLE I.—*Percentage of soluble and total boron in soil samples, Acampo, Cal., 1915*

Boric acid added to upper 6 inches of soil.	Soluble boron as boric acid found.	Total boron as boric acid found.
Per cent.	*Per cent.*	*Per cent.*
0.00176 as borax	0	0.00003
0.00232 as colemanite	0	.00001
Control	0	.00001

BARLEY EXPERIMENTS

The effect of borax and colemanite on the growth of barley (*Hordeum* spp.) was studied at Walnut Creek, Cal., by Mr. Nougaret. In these experiments the manure was also applied at the rate of 16 tons per acre. Three plots were used for the test, one fertilized with borax-treated manure, one with colemanite-treated manure, and a third with untreated

manure. The amount of borax and colemanite added to the manure is given on page 451.

No injurious effect of the boron was noticeable during the growing period. The yields were not determined, but so far as could be estimated, the borax and colemanite did not affect the yields. The crop was harvested in June, 1915. At the time of harvest samples of grain and straw from each plot were analyzed for solids, nitrogen, and boric acid. The results are given in Table II.

TABLE II.—*Percentage of solids, nitrogen, and boron in barley, grain, and straw, dry basis, Walnut Creek, Cal., 1915*

Boric acid added to upper 6 inches of soil.	Material.	Solids.	Nitrogen.	Total boron as boric acid found.
Per cent.		*Per cent.*	*Per cent.*	*Per cent.*
0.00176 as borax	Straw	93.97	0.34	0
	Grain	91.75	1.52	.00001
0.00232 as colemanite	Straw	93.26	.27	.00001
	Grain	90.00	1.46	.00002
Control	Straw	91.78	.41	.00001
	Grain	91.75	1.59	0
Control (between rows)	Straw	92.44	.52	.00002
	Grain	91.93	1.43	.00001

The nitrogen in the grain was practically equal in all samples, while the two samples of straw grown on the boron-treated plots contained less nitrogen than the two control straw samples. Only minute amounts of boron were found in the barley, either straw or grain. The control samples showed the presence of as much boron as there was in the barley grown on soil to which boron was added. No injurious action of the added boron was apparent.

In this connection the difference in the effect of boron in nutrient solution as compared with that in field experiments is illustrated by the findings of Brenchley (1) at the Rothamsted station. She found boric acid to be definitely poisonous to barley down to 1 part in 250,000.

Samples of soil were taken from each of the three plots and analyzed for soluble and total boron. The results are given in Table III.

TABLE III.—*Percentage of soluble and total boron in soil samples from Walnut Creek, Cal., 1915*

Boric acid added to upper 6 inches of soil.	Soluble boron as boric acid found.	Total boron as boric acid found.
Per cent.	*Per cent.*	*Per cent.*
0.00176 as borax	0	0.00008
0.00232 as colemanite	0	.000024
Control	0	.00004

More total boron was present in the control sample than was found in one of the two treated samples. The control showed four times as much boric acid as the control from the peach orchard at Acampo, Cal.

WHEAT EXPERIMENTS

Similar experiments were tried testing the effect of borax and colemanite on the growth of wheat (*Triticum* spp.) at Benton Harbor, Mich., during the season of 1914–15. The same amounts of borax and colemanite were added as in the two previous tests. Mr. F. L. Simanton, of the Bureau of Entomology, directed these experiments. No effect of the boron was evident during the growth of the crop; nor were the yields influenced by the added boron. Samples of grain and straw were taken from all three plots in June, 1915, and analyzed for solids, nitrogen, and boric acid. Samples of wheat stubble were also analyzed. The results are given in Table IV.

TABLE IV.—*Percentage of solids, nitrogen, and boron in wheat grain and straw, dry basis, Benton Harbor, Mich., 1915*

Boric acid added to upper 6 inches of soil.	Material.	Solids.	Nitrogen.	Total boron as boric acid found.
Per cent.		*Per cent.*	*Per cent.*	*Per cent.*
0.00176 as borax	Straw	94.04	0.42	0.00267
	Grain	89.40	1.97	.0008
0.00232 as colemanite	Straw	93.07	.52	.00016
	Grain	89.76	2.15	.00016
Control	Straw	92.84	.35	.00040
	Grain	88.91	1.69	.00012
0.00176 as borax	Stubble, second growth	94.17	.....	.0020
	Roots of stubble, second growth.	94.64	.....	.00036
0.00232 as colemanite	Stubble, second growth	94.47	.....	.0040
	Roots of stubble, second growth.	96.35	.....	.00001
Control	Stubble, second growth	94.42	.....	.00001
	Roots of stubble, second growth.	94.90	.....	.00001

There was less nitrogen in the grain and straw from the control plot than from the boron-treated plots. The grain contained less boric acid than the straw. It is apparent that the wheat, both grain and straw, took up more boron from the borax-treated than from the colemanite-treated plot. The roots of the stubble contained much less boric acid than the tops. The amount of boric acid in the stubble from the control plot was much less than in the control straw of the first growth of wheat on the same plot.

At the time the wheat samples were taken soil samples were also obtained. Analyses of these samples are reported in Table V.

TABLE V.—*Percentage of boron in soil samples from Benton Harbor, Mich., 1915*

Boric acid added to upper 6 inches of soil.	Soluble boron as boric acid found.	Total boron as boric acid found.
Per cent.	*Per cent.*	*Per cent.*
0.00176 as borax	0	0.00004
0.00232 as colemanite	0	.00012
Control	0	.00003

As in the other samples of soils analyzed, no soluble boron was present. More total boric acid was found in the sample from the colemanite than in the sample from the borax plot. The amount of boric acid in the control soil was practically the same as found in the control plot of the barley experiments at Walnut Creek, Cal.

EFFECT OF TWO APPLICATIONS OF BORON-TREATED MANURE ON VEGETABLES

Nine plots, each 29.7 by 40.74 feet, with 3-foot guards were used for these experiments at Arlington Experimental Farm. Three of the plots received manure containing added borax; three of them received similar applications of manure containing added colemanite; while the remaining three plots received untreated manure. The amounts of boron added to the plots are given on page 451.

EXPERIMENTS OF THE FIRST SEASON, 1914

Six rows of spinach (*Spinacia oleracea*) and six rows of kale (*Brassica oleracea acephala*) per plot were planted on October 1, 1914. On this date young cabbage (*Brassica oleracea capitata*) and lettuce (*Lactuca sativa*) plants, two rows each per plot, were set out. On October 9 it was evident that the boron had not prevented germination, as spinach and kale plants appeared above the ground on all plots. The lettuce and cabbage plants were normal. On November 10 it was evident that the spinach and kale plants were not as numerous on all the 24- and 40-ton plots as on the 16-ton plots. No signs of burning were observed on any of the plots. No injurious action of the boron was evident in the early spring of 1915. The majority of the lettuce and cabbage plants did not survive the winter, but the spinach and kale looked normal.

ANALYSES OF VEGETABLES AND SOIL

On June 26, 1915, lettuce, cabbage, spinach, and kale plants, also samples of the soil 6 inches deep, were taken from the different plots. The results of the analyses of the vegetables are given in Table VI, and the soil results in Table VII. The methods used were those described on page 452. The tops of all varieties of the plants contained more boron when grown on the 40-ton plots than when grown on the 16- or 24-ton plots. All the roots gave negative results for boron. More boron was

absorbed by the plants grown on the 40-ton colemanite-treated plot when the boron was added as the insoluble calcium colemanite than when grown on the 40-ton borax plot where the boron was added in a readily soluble form. The control samples tested were remarkedly free from boron, only the tops of the kale showing any traces. The flowering tops of the kale contained about the same amount of boron as the rest of the tops of the plant. Nakamura (7) found that 0.0001 per cent of boric acid in culture solutions stimulated spinach, but had a slightly injurious action on peas.

Five samples of soil, two from each of the light and the heavy boron-treated plots and a control sample, were analyzed for nitrates, ammonia, and soluble boron.

TABLE VI.—*Percentage of the total boron as boric acid in lettuce, spinach, cabbage, and kale, dry basis, Arlington, Va., 1914–15*

Boric acid added to upper 6 inches of soil.	Parts of plants analyzed.	Boric acid found.			
		Lettuce.	Spinach.	Cabbage.	Kale.
Per cent.		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.00176 as borax (16-ton plot).	Tops	.....	0	Trace	0
	Roots	.....	0	0	0
	Flowering tops	.....	.....	.....	.00025
0.00264 as borax (24-ton plot).	Tops	Trace	.....	0	.....
	Roots	0	.....	0	.....
	Flowering tops	.....	.....	.....	.....
0.0044 as borax (40-ton plot).	Tops	.0002	.002	Faint trace.	.001
	Roots	0	0	0	0
	Flowering tops	.....	.....	.....	.0014
0.00232 as colemanite (16-ton plot).	Tops	0	.001	.004	.....
	Roots	0	0	0	.....
	Flowering tops	.....	.....	.....	.....
0.00348 as colemanite (24-ton plot).	Tops	0	.....	.00006	.....
	Roots	0	.....	0	.....
	Flowering tops	.....	.....	.....	.....
0.0058 as colemanite (40-ton plot).	Tops	.00225	.0085	.00475	.00625
	Roots	0	0	0	0
	Flowering tops	.....	.....	.....	.00365
40-ton control	Tops	.....	0	0	.0001
	Roots	.....	0	0	0
	Flowering tops	.....	.....	.....	0

TABLE VII.—*Percentage of nitrate and ammonia nitrogen and soluble boron in soil samples, Arlington, Va., 1915*

Boric acid added to upper 6 inches of soil.	Nitrate nitrogen.	Ammonia nitrogen.[1]	Soluble boron as boric acid found.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
0.00176 as borax (16-ton plot)	0.00173	0.0016	0
0.0044 as borax (40-ton plot)	.01106	.0023	0
0.00232 as colemanite (16-ton plot)	.00128	.0016	0
0.0058 as colemanite (40-ton plot)	.00216	.0021	0
40-ton control	.00263	.0017	0

[1] Polin method.

It is evident from the negative soluble boron results that the boron is combined in an insoluble form in the soil, although much of it is undoubtedly held in levels lower than the first 6 inches of soil The nitrate results for the soil from the boron-treated plots are, with the exception of the 40-ton borax-treated plot, a little lower than in the control plot. The amount of ammonia in the boron-treated samples averages higher than that in the control soil.

EXPERIMENTS OF THE SECOND SEASON, 1915

Soon after the samples had been taken from the plots on June 26, 1915, the ground was again fertilized with manure treated as before—that is, the same amounts of boron-treated and untreated manure were added to their respective plots and it was plowed under and the ground harrowed and rolled. On July 15 corn (*Zea mays*), turnips (*Brassica rapa*), and pea beans (*Phaseolus vulgaris*) were planted. On August 10 it was apparent that the beans grown on the plots to which boron-treated manure had been added had been severely injured. The turnips on the 16-ton boron-treated plots were normal, but were thinner on the 24- and 40-ton boron-treated plots than on the corresponding controls. The growth of corn was irregular on all the plots, but no yellowing from the boron was apparent. The beans showed a yellowing on all the plots. The turnips that came up on the 24- and 40-ton plots showed no yellowing. The growth of all the vegetables was poor on all the 40-ton plots, and a marked reduction in the crop yield was evident on the 24-ton plots, especially where boron had been added to the soil. On September 7 the plants on the 16-ton plots were in a fairly good condition. The only injury to any of the plants on these plots that may be attributed to the boron was a yellowing of the bean plants. On the 24-ton plots none of the crops were doing well, although the control was undoubtedly the best of the three plots. The beans were injured and the growth of the turnips was irregular, especially on the borax-treated plot. The corn showed a reduced stand as one effect of the added boron. The 40-ton boron treatments had injured all the crops, the beans severely, the turnips and corn considerably. The crops on the 40-ton control plot were poor and showed some injury.

One row (the middle row) of corn and of beans were pulled from each plot and weighed by Mr. Rhodes, of the Arlington farm, in October, 1915. These weights, given in Table VIII, represent the whole plants, including the roots.

The weights of beans and corn given in Table VIII indicate a greater influence of the 24-ton boron-treated manure plot than was evident from the observations reported above. While the amount of boron in the manure did not produce any outward evidence of injury on corn, it seriously affected the yield. It is also evident that the untreated manure

when added at the 24-ton rate reduced the yield markedly over the yield on the plots fertilized at the 16-ton rate. With the control the reduction of the 24-ton over the 16-ton plot was proportionately greater than with the 40-ton over the 24-ton plot. With the boron-treated plots the marked action of the 40-ton applications is shown by the practical destruction of the bean crop and the marked reduction of the yield of corn.

TABLE VIII.—*Weights of one row of whole bean and corn plants at Arlington Experimental Farm, 1915*

Boric acid added to upper 6 inches of soil.	Beans.		Corn.
Per cent.	*Lb.*	*Oz.*	*Lb.*
0.00176 as borax (16-ton plot)	3	1	37
0.00264 as borax (24-ton plot)	2	12	17
0.0044 as borax (40-ton plot)	0	9	14
0.00232 as colemanite (16-ton plot)	12	9	54
0.00348 as colemanite (24-ton plot)	3	5	21
0.0058 as colemanite (40-ton plot)	0	5	15
16-ton control	15	1	48
24-ton control	9	10	29
40-ton control	7	4	30

On October 5 samples of turnips from the 16- and 40-ton borax-treated manure plots and from the 40-ton control plot were collected and tested with the results given in Table IX.

TABLE IX.—*Percentage of boron in turnips, dry basis, at Arlington Experimental Farm, 1915*

Boric acid added to upper 6 inches of soil.	Parts of plants analyzed.	Total boron as boric acid found.
Per cent.		*Per cent.*
0.00176 as borax (16-ton plot)	Tops	0.0036
	Roots	.0012
0.0044 as borax (40-ton plot)	Tops	.0032
	Roots	.0017
40-ton control	Tops	.0020
	Roots	.00002

The tops contained the greater portion of the boron. The boron in the roots varied directly with the boron added to the soil, whereas in the tops this was not the case. On comparing these results with the results reported in Table VI for the plants grown on these plots after the first application of manure containing added boron, it is evident that no more boron was taken up by the turnips grown after the second application of boron-treated manure than by the plants after the first application.

Samples of soil were taken from these plots on October 5 and tested for solids, nitrate and ammonia nitrogen, and total and soluble boron. The results are given in Table X.

TABLE X.—*Analyses of soil samples from Arlington Experimental Farm, 1915*

Boric acid added to upper 6 inches of soil.	Solids.	Nitrogen as nitrates.	Nitrogen as ammonia (Folin method).	Soluble boron as boric acid found.	Total boron as boric acid found.
Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.00176 as borax (16-ton plot)	82.43	0.0049	0.00042	0	0.00010
0.0044 as borax (40-ton plot)	82.13	.0056	.00083	0	.00014
40-ton control	79.85	.0051	.00083	0	.00005

No effect which may be attributed to the boron is evident from the results given in Table X. It is known that in cases of excessive applications of manure, as in the two 40-ton applications reported above, normal bacterial metabolism does not take place. This may account for the lack of evidence of the influence of the boron on the nitrogen constituents of the soil. No soluble boric acid and only small amounts of total boric acid were found in the soil. The absence of soluble boron is in agreement with the first year's findings.

It is evident that there is no accumulation of boron in the upper 6 inches of soil following two heavy applications of borax to the soil within one year.

EFFECTS OF THREE APPLICATIONS OF MANURE CONTAINING BORON ON CROPS AND SOIL

Experiments to determine the effect of three applications of boron-treated manure on crops and soil were carried out on the farm of the Bureau of Animal Industry at Bethesda, Md., in 1914, 1915, and 1916. A plot of ground 206 feet long and 60 feet wide was selected. The experiments were similar to those at the Arlington Experimental Farm and included three successive applications of horse manure containing added borax and of manure containing added colemanite to the same soil and their influence on the crops grown thereon. Nine plots were used, and the treated and untreated manure was applied at the rate of 16, 24, and 40 tons per acre. The amount of boron added is given on page 451. Guards 3 feet wide separated all the plots. The distribution of the boron in different parts of the crops grown on the various plots and its effect on the nitrogen distribution in the soil were also studied.

EXPERIMENTS OF THE FIRST YEAR, 1914

On October 1, 1914, kale, lettuce, and spinach seeds and onion (*Allium cepa*) sets were planted in all the plots. Notes taken at different times following the planting showed that all plots looked equally well. In April, 1915, it was seen that the lettuce had not survived the winter but the kale, spinach, and onions were growing. No injury and no variation in the growth on any of the plots were seen. It was apparent that the

boron, either as borax or as calcined colemanite, had exerted no ill effects on the growth of these crops. Samples of onions were taken from the various plots for analysis for boron in July, 1915. They had gone to seed; and the flowering tops, as well as the tops and roots, were tested. The flowering tops from the borax treated plot, where the manure had been applied at the 40-ton rate showed 0.00004 per cent of boric acid. The rest of the plants or portions of plants gave negative results for boron. Jay (*6*) reported that he found considerable amounts of boron in onions. As will be seen later, none of the plants grown at Bethesda absorbed much boron.

EXPERIMENTS OF THE SECOND YEAR, 1915

The plots were cleared, and a second application of manure was made to all plots on July 1, 1915, with the same quantities of borax and colemanite and three different rates of manure application as the first year—that is, the plots received exactly the same amounts of boron as in September, 1914. The manure was harrowed into the ground, not plowed, so as to have the boron in the upper 3 or 4 inches of soil in order to obtain the maximum effects. On July 12, 1915, Golden Bantam corn, green stringless beans, table beets (*Beta vulgaris*), and McCormick potatoes were planted. On July 29 the corn, beets, and beans were up, and a potato plant was seen here and there. The beans showed a yellowing on the borax-treated and colemanite-treated plots, even where the manure was applied at the 16-ton rate. During August the beans growing on the 40-ton borax-treated plots showed decided injury, while those on the 16- and 24-ton plots showed some injury. Colemanite prodiuced much less injury to the beans than the borax. There were fewer potato plants on the plot where borax manure was applied at the 40-ton rate than on the other plots. It was very noticeable during August that no weeds were growing on the colemanite plots, while weeds grew luxuriantly on the control and the borax treated plots.

On August 20 the beets, as well as the beans, showed that they had been injured by the borax. The corn on the control plots had the highest stand, but no injury was apparent to the corn on the borax or colemanite plots. The potatoes on the 40-ton borax-treated plot showed a slight yellowing of the leaves. The potatoes on the other plots were apparently normal.

On this date the beans on the 16-ton borax-treated plot had outgrown the initial injury, and the three other crops on this plot were normal. The colemanite-treated manure at the 16-ton rate caused no injury to any of the vegetables. On the 16-ton control plot all the plants were normal, but the beets were thinner than on the 16-ton colemanite-treated plot.

TABLE XI.—*Percentage of total boron as boric acid in potatoes, corn, beets, and string beans, dry basis, Bethesda, Md., 1915*

POTATOES

Boric acid added to upper 6 inches of soil.	Material.	Solids.	Total boron as boric acid found.
Per cent.		*Per cent.*	*Per cent.*
0.00176 as borax (16-ton plot)	Tops	.....	Trace.
	Roots	.....	0.000320
	Tubers	19.11	.000004
0.00264 as borax (24-ton plot)	Tops	.....	.001000
	Roots	.....	.000280
	Tubers	17.01	.000006
40-ton control plot	Tops	.....	.000500
	Roots	.....	.000400
	Tubers	15.51	.000004

CORN

0.00176 as borax (16-ton plot)	Stalk	.....	0
	Ears [1]	85.5	.00004
	Roots	.....	0
0.00264 as borax (24-ton plot)	Stalk	.....	0
	Ears	66.74	.00007
	Roots	.....	0
40-ton control plot	Stalk	.....	0
	Ears	84.36	.00005
	Roots	.....	0

BEETS

0.00176 as borax (16-ton plot)	Tops	.....	0
	Roots	11.95	.000002
0.00264 as borax (24-ton plot)	Tops	.....	0
	Roots	13.45	.000022
40-ton control plot	Tops	.....	0
	Roots	10.36	.000012

STRING BEANS

0.00176 as borax (16-ton plot)	Beans and pods	11.39	0.00004
	Tops	18.77	.00040
	Roots	27.44	.00006
0.0044 as borax (40-ton plot)	Beans and pods	12.63	.00001
	Tops	21.06	.00003
	Roots	32.08	.00006
0.00232 as colemanite (16-ton plot)	Beans and pods	10.31	.00001
	Tops	17.78	.00012
	Roots	30.49	.00004
0.0058 as colemanite (40-ton plot)	Beans and pods	10.41	.00001
	Tops	19.04	.00001
	Roots	28.26	.00001
16-ton control plot	Beans and pods	9.04	.00002
	Tops	19.81	.00008

[1] Nitrogen results=1.39, 1.57, and 1.18 per cent, respectively.

The 24-ton borax treatment decidedly injured both beets and beans, but no injury to corn or potatoes was apparent. On the 24-ton colemanite-treated plot beans were injured considerably and beets slightly, while corn and potatoes were normal.

The 40-ton borax treatment injured all the plants; beans and beets severely and corn and potatoes slightly. The 40-ton colemanite treatment injured beans and beets only. The 40-ton control plot did not produce as good crops as the other control plots.

The corn on all control plots showed the highest stand, although the other plots showed no apparent injury. The potatoes showed no injury, except a slight yellowing of the leaves on the plants grown on the 40-ton borax-treated plot.

During October, 1915, the crops were harvested and samples from each plot were taken for analysis. Soil samples 6 inches deep were also taken from each plot at this time. The potatoes from the three borax-treated plots made 3¼ bushels, from the three colemanite-treated plots 2¼ bushels, and from the three controls 2⅛ bushels. Analyses of potatoes, corn, beets, and string beans from several of the plots are given in Table XI. It is evident from this table that the plants grown on the 24- and 40-ton borax-treated plots took up slightly more boron than those grown on the 16-ton borax-treated plot. The roots and tops of the potatoes contained more boron than the tubers in all the samples tested. The corn showed the presence of boron in the ears only. All of the beet samples analyzed showed the presence of minute amounts of boron in the roots, but none in the tops. The string beans and pods were analyzed together. Separate analysis of tops and roots were made. The tops contained more boron than the roots, and the beans and pods the least. The beans did not seem to take up the boron in proportion to the amounts added to the soil, as less boron was found in the plants grown on the 40-ton than in those on the 16-ton plots. The amounts found were small in all cases, owing to the fact that the samples were taken in October and the severely injured plants, which contained the comparatively large amounts of boron, had died earlier.

Peligot (*8*), in 1876, tested the influence of boron on beans. He used borax, boric acid, and potassium borate, 2 gm. per liter. The leaves turned yellow and all the plants were killed.

Haselhoff (*5*) grew beans in culture media containing borax and boric acid. A spotting of the leaves was observed, but this does not necessarily indicate an injury to plant growth. Boron was detected in the straw, but not in the beans.

The figures for solids in beans and pods showed higher results for the treated than for the control samples.

THIRD YEAR OF THE EXPERIMENTS, 1916

The beets, corn, beans, and potatoes were removed during October, 1915, and the ground was prepared for a third application of manure containing added borax and colemanite. The amount of manure and the treatments were identical with the two previous ones. After the manure had been applied to the plots, they were plowed and harrowed. On November 3 rye was planted on all the plots and the ground re-harrowed.

On November 29 the rye had germinated and appeared 2 inches above the ground. The rye on the control plots was not affected by the heavy applications of manure, but on the colemanite-treated plot, 40-ton application, the rye showed a red tinge at the base of the leaf and stems. The tips of the leaves were green. The plants on the 24-ton colemanite-treated plot showed this reddish tinge to a slight degree, while the plants on the 16-ton plot were normal. On the borax-treated plot, 40-ton rate, a slight reddish coloration of the leaves was observed. This was not quite as noticeable as on the corresponding colemanite-treated plot. Plants on the 16- and 24-ton borax-treated plots were normal.

During the spring of 1916 the initial coloration of some of the plants on the heavily boron-manured sections disappeared, and throughout the growing period in the spring it was impossible to detect any difference in the rye on the various plots. It was found necessary on harvesting the rye on June 27 to cut it with a scythe, as the wind and rain had beaten down a great deal of it. The rye from each plot was stacked separately, and 100 heads were picked at randon from each of the nine plots. The total weights of the straw and grain from each plot were also taken. The weights are given in Table XII, together with analyses of the rye heads and rye straw from each plot for moisture, fat, nitrogen, and boric acid. It is evident from the weights of straw and grain that the best yields were obtained from the 16-ton plots, while the 40-ton plots gave the lowest yields. The total weight of the straw from the three plots receiving the same treatment showed but little difference—that is, from the borax-treated plots 820 pounds of straw and grain were obtained; from the colemanite plots 725 pounds (on two of the plots the straw was beaten down), and from the three control plots 800 pounds (the straw was beaten down on one of the three plots). The striking fact is that the yields from the three control plots showed the same tendency to decrease with an increased application of manure as from the boron-treated plots, which indicates that the increased amounts of manure rather than the boron were the determining factors. The weights of the 100 heads of rye given in Table XII are slightly larger for the control samples than for the heads grown on either the borax or the colemanite manured plots. The weight of 100 heads from the 40-ton borax-

treated plot was greater than from the 16- or 24-ton borax-manure plots, while in the colemanite and control plots the weights of 100 heads from the three plots of each series were very uniform. It is evident that the grain from the control plots was better filled out than from the other plots, and an examination of a large number of heads from all plots proved this to be so.

In brief, the fact that the heads of rye were not filled as well from the two series of boron-manured plots as from the controls and the slight red coloration of the plants during the first few weeks of growth are the only evidences obtained of any injurious action of boron. The total yields of straw and grain appeared to be influenced by the amounts of manure rather than by the boron added to the soil, the yields from the three plots heavily treated with manure being much lower than from the three plots receiving light applications of manure.[1]

TABLE XII.—*Analyses of rye, dry basis, Bethesda, Md., 1916*

Boric acid added to upper 6 inches of soil.	Weight of 100 heads.	Moisture.	Fat.	Nitrogen.	Total boron as boric acid found.
HEADS					
Per cent.	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
0.00176 as borax (16-ton plot)	128.4	9.27	1.34	2.05	0.0001
0.00264 as borax (24-ton plot)	141.1	9.30	1.38	2.24	.0002
0.0044 as borax (40-ton plot)	151.2	9.31	1.34	2.46	.0006
0.00232 as colemanite (16-ton plot)	141.0	9.31	1.37	2.18	.0004
0.00348 as colemanite (24-ton plot)	138.0	9.32	1.29	2.22	.0003
0.0058 as colemanite (40-ton plot)	141.0	9.30	1.41	2.22	.0004
16-ton control plot	157.5	9.28	1.24	1.95	.0001
24-ton control plot	152.3	9.31	1.34	2.04	.0001
40-ton control plot	158.6	9.34	1.26	2.27	.00015
STRAW					
	Pounds.				
0.00176 as borax (16-ton plot)	315	9.41	1.05	0.93	0.00055
0.00264 as borax (24-ton plot)	265	9.45	.83	1.23	.00110
0.0044 as borax (40-ton plot)	240	9.41	1.04	1.39	.00196
0.00232 as colemanite (16-ton plot)	340	9.48	.97	.98	.00022
0.00348 as colemanite (24-ton plot)	[a] 210	9.49	.99	1.10	.00035
0.0058 as colemanite (40-ton plot)	175	9.48	1.07	1.15	.00080
16-ton control plot	340	9.48	1.20	1.11	Trace.
24-ton control plot	300	9.53	1.22	1.08	.0001
40-ton control plot	[a] 160	9.49	1.24	1.25	.0002

[a] Straw beaten down by wind and rain.

The moisture and fat results for the rye heads reported in Table XII are very uniform. The nitrogen results show a decided rise with the increased amounts of manure added to the borax-manure and control plots. More boric acid was found in the rye heads grown on the 40-ton

[1] The assistance of Mr. R. H. Hutchison, of the Bureau of Entomology, in supervising the planting and harvesting of the rye and in reporting the findings to the writer is greatefully acknowledged.

borax-treated plot than on the 16- or 24-ton plots. The amounts of boric acid found in the heads from the colemanite-manure plots were practically the same in the three cases. Small quantities of boric acid were found in the heads from all three of the control plots.

Similar determinations are given for the straw taken from all of the plots. The moisture results are very uniform for the nine samples, while the fat figures are a little higher for the three control samples than where boron was added to the soil. The nitrogen results show a marked increase, varying directly with the amounts of manure applied to the soil. The results for boric acid show that the rye took up the boron from the different plots in proportion to the quantities of boron added to the soil. On the other hand, the form in which the boron was added had a decided influence on the amounts absorbed by the plants, over twice as much boron of the readily soluble borax being absorbed by the plants as when the boron was added as the insoluble colemanite (calcium borate).

The control samples showed the presence of small amounts of boron.

ANALYSES OF SOIL, BETHESDA, MD., 1914–15

Samples of soil were taken 6 inches deep from the different treated and untreated plots. The samples were taken three times—that is, just prior to the second and third applications of manure and at the time of harvesting the third crop, eight months after the third application of the manure to all plots. The total nitrogen and the volatile nitrogen results obtained on distilling with magnesium oxid (see Table XIII) vary considerably for the different years, but the samples taken from all the plots on the same dates are rather uniform. The results for volatile nitrogen by the Folin aeration method are higher in the samples taken on June 1, 1915, after the first addition of manure to the soil, than in the samples taken on September 3, following the second addition, or in the samples taken on June 1, 1916, after the third manure treatment. The reduction in the control samples makes it evident that the boron was not the cause of the decrease. The nitrate results show the same tendency to decrease in each succeeding set of samples from June, 1915, to June, 1916, as was noted before for ammonia. There is no apparent reduction in either the ammonia or nitrates from the added boron.

No acid-soluble boric acid was found in any sample. The largest amounts of total boric acid were found in the samples taken on September 3, 1915, only two months after the second application of the manure, while with the samples taken on June 1, 1915, and June 1, 1916, seven months had elapsed since the addition of the boron-manure to the soil. No more total boric acid was found in the soil samples taken in June, 1916, after the third application of boron-manure than in the samples taken in June, 1915, following the first application of boron-manure to the plots. In view of this, and also as the most boron was found in the

samples taken on September 3, 1915, two months after the second addition of boron-manure to the soil, it is evident that the boron gradually disappeared from the upper 6 inches of soil, and no cumulative action of boron resulted from the three successive additions of boron-treated manure to the same soil when 0.08 pound of borax or 0.095 pound of colemanite was added to the bushel and the manure applied to the soil at a rate of 40 tons per acre.

TABLE XIII.—*Nitrogen distribution and boron as boric acid in the soil, Bethesda, Md.*

[Samples of soil were taken from same plots following each of the three additions of manure and boron]

Treatment of soil.	Boric acid added to upper 6 inches of soil.	Nitrogen determined as—				Boron as boric acid found.	
		Total.	Ammonia (MgO method).	Ammonia (Folin method).	Nitrates.	Soluble.	Total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
40-ton borax-treated plot [1]	0.0044	0.161	0.014	0.00070	0.00324	0	0.000112
40-ton colemanite-treated plot [1]	.0058	.161	.014	.00050	.00135	0	.00008
40-ton control-treated plot [1]	0	.154	.014	.00100	.00232	0	.000024
16-ton borax-treated plot [2]	.00176	.119	.042	.00033	.00282	0	.00048
40-ton borax-treated plot [2]	.0044	.168	.042	.00050	.00286	0	.00048
16-ton colemanite-treated plot [2]	.00232	.119	.042	.00063	.00116	0	.00040
40-ton colemanite-treated plot [2]	.0058	.168	.028	.00066	.00208	0	.000024
16-ton control-treated plot [2]	0	.119	.042	.00066	.00156	0	.00012
16-ton borax-treated plot [3]	.00176	.140	.014	.00060	.00091	0	.00008
24-ton borax-treated plot [3]	.00264	.204	.014	.00060	.00144	0	.00012
16-ton colemanite-treated plot [3]	.00232	.158	.014	.00050	.00100	0	.00004
40-ton colemanite-treated plot [3]	.0058	.196	.010	.00060	.00030	0	.00012
24-ton control-treated plot [3]	0	.189	.014	.00030	.00130	0	.00004

[1] Samples taken on June 1, 1915. Manure applied in October, 1914.
[2] Samples taken on September 3, 1915. Manure last applied in June, 1915.
[3] Samples taken on June 1, 1916. Manure last applied on November 1, 1915.

SECOND ANALYSES OF BORON-TREATED SOILS FROM THE SOUTH

In order to obtain information as to the length of time that boron remained in the soil, a second series of samples of soil from Orlando, Fla.; Dallas, Tex.; and New Orleans, La., were analyzed in June, 1916. These soils had received applications of manure plus borax and plus colemanite the year previous, and analyses of the soils for soluble boron have been reported (*2*). The results of the analyses are given in Table XIV.

In 1915 soluble boron was found in all of the Orlando and New Orleans samples and in two of the Dallas soil samples. In 1916 no soluble boron was found in the samples from any of the boron-manure or control plots. Only relatively small amounts of total boron were found in these soils in 1916. The samples from the boron-treated plots showed the presence of only a little more boron than the controls. The disappearance of the boron and the holding of the small amount present in an insoluble form is a remarkable demonstration of the power of the soil to absorb toxic substances and to hold them in an inactive form.

TABLE XIV.—*Percentage of total and soluble boron, nitrogen, and nitrates found in samples of soil from Orlando, Fla.; Dallas, Tex.; and New Orleans, La.*

Soils analyzed.	Soluble boron as boric acid.	Total boron as boric acid.	Total nitrogen.	Nitrogen as nitrates.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Orlando, Fla.:				
Borax plot [1]	0	0.00003	0.056	0.00572
Control plot	0	.00002	.056	.00777
Dallas, Tex.:				
Borax plot (light)[2]	0	.00001	.208	.00667
Borax plot (heavy)[2]	0	.00001	.295	.00805
Control plot	0	0	.253	.00800
New Orleans, La.:				
Borax plot [2]	0	.000040	.084	.00084
Control plot	0	.000024	.070	.00090

[1] 0.75 pound of borax added per 8 bushels of manure; manure applied at 16-ton rate.
[2] 1.25 pounds of borax added per 8 bushels of manure; manure applied at 16-ton rate.

SUMMARY

The influence of single applications of horse manure containing borax or colemanite added at the respective rates of 0.08 and 0.095 pound to the bushel and applied to the soil at the rate of 16 tons per acre was tested on peach trees at Acampo, Cal., on barley at Walnut Creek, Cal., and on wheat at Benton Harbor, Mich. The upper 6 inches of soil on the borax-treated plots were calculated to have contained 0.00176 per cent of boric acid and that on the colemanite plots 0.00232 per cent.

No influence on the growth or yield of wheat or barley was observed, yet the boron seemed to have a beneficial effect on peach trees. There was no soluble boron and but little total boron in any of the soil samples from the three different localities.

Experiments were conducted at Arlington, Va., and Bethesda, Md., in which the horse manure treated with borax and colemanite was applied as noted above, but at three different rates per acre—that is, 16, 24, and 40 tons. The tests at the Arlington Experimental Farm extended over two seasons and at Bethesda over three seasons. The soil was calculated to have contained in the upper 6 inches when borax-manure was applied at the 16, 24, and 40 ton rates, 0.00176, 0.00264, and 0.0044 per cent of boric acid, respectively, and 0.00232, 0.00348, and 0.0058 per cent respectively, when colemanite was applied.

The first season at Bethesda 0.0044 per cent of boric acid as borax and 0.0058 per cent as colemanite caused no injury to lettuce, spinach, kale, or onions. These percentages of boric acid the first season at the Arlington farm caused a reduction in crop with lettuce, spinach, kale, and cabbage.

At Arlington 0.00264 per cent of boric acid as borax and 0.00348 per cent as colemanite reduced the yield of spinach and kale by preventing germination. The difference in the action of the same percentages of

borax and colemanite on the same crops at Arlington and Bethesda is rather striking. The colemanite and borax showed no difference in action on plants grown in the same soil the first season. More boron was found in the lettuce, cabbage, spinach, and kale plants grown on the 40-ton colemanite-treated plots than in the plants from the 40-ton borax-manure plots at Arlington. Onions grown at Bethesda showed the presence of boron only in the flowering tops of the plants grown on the 40-ton borax-treated plot.

The second season 0.00176 per cent of boric acid as borax injured string beans at both Arlington and Bethesda; 0.00232 per cent as colemanite at Arlington injured string beans, while at Bethesda this percentage of colemanite caused no injury; but 0.00348 per cent of boric acid as colemanite injured string beans at Bethesda. At Arlington 0.00264 per cent of boric acid as borax and 0.00348 per cent as colemanite reduced the yield of corn and turnips, but at Bethesda were apparently without effect on the yield of corn. At Bethesda 0.0044 per cent of boric acid as borax and 0.0058 per cent as colemanite reduced the yield of potatoes and corn.

The potatoes, corn, beets, and string beans grown at Bethesda contained but small amounts of boron. The turnips at Arlington contained more boron than the plants at Bethesda. It is evident that the vegetables took up more boron from the soil at Arlington than at Bethesda.

Rye was grown the third season at Bethesda following the third application of boron manure. A reddish tinge was observed in the young plants on the 40-ton boron-treated plots. This disappeared gradually, and in the spring the rye looked normal The rye heads grown on the boron plots were not as well filled out as the heads of the plants on the control plots. The crops on the 24- and 40-ton control plots were materially reduced by the large amounts of manure applied. The quantity of manure added was of more importance in reducing the yield than the added boron.

There is a decided difference in soils in rendering the added boron nontoxic to plants. This is seen in the divergent results as to plant injury, etc., obtained on adding equal amounts of borax or colemanite to different soils. In some cases boron is taken up by plants from soil when no detectable quantities of boron are present in the soil samples.

There is a complete disappearance of detectable amounts of soluble boron from soils after the addition of borax and colemanite, although small amounts of total boron are present. It is therefore evident that insoluble boron compounds are formed. In many soils there is a tendency for plants to absorb boron in proportion to the quantities added. In some soils the same amounts of boron were absorbed irrespective of the quantities added. The calcium of the colemanite did not prevent the

absorption of boron, although usually more boron was absorbed by the plants when the boron was added as borax than as colemanite. The amount of boron absorbed by plants depends on the character of the soil more than on the form in which the boron was added.

The absorption of boron by plants varies with the variety of plant, the solubility of the boron compound, the quantity of the boron compound added to the soil, the time elapsing after the compound is mixed with the soil before planting, the amount of rainfall, etc., and finally with the character of the soil to which the boron compound is added.

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DESTRUCTION OF TETANUS ANTITOXIN BY CHEMICAL AGENTS

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OBJECT AND PLAN OF WORK

The ultimate object of the work herein described is a solution of the problem of the chemical nature of antitoxins and their preparation in the pure state. That this would be attained was not expected, in view of the numerous previous investigations which left these problems unsolved. But it seemed highly probable that data would be obtained which would throw some light on the subject and serve as guides for other investigations.

Up to the present time numerous investigators have attempted to separate antitoxins from their associated proteins, but without complete success. The well-known tetanus and diphtheria antitoxins are examples of preparations containing all or nearly all of the immunity units present in the original serums, but only a part of the proteins. Thus, Homer (8)[1] concentrated a tetanus serum containing 100 units per cubic centimeter and 6 per cent of protein, obtaining a product that contained 900 units per cubic centimeter and 19 per cent of protein. In this process 10 per cent of the antitoxic units were lost, the final product was 9 times as potent as the original serum and contained but 3 times as much protein. The failure of all attempts to obtain a protein-free antitoxin preparation has led some investigators to the conclusion that the antibody (or group of antibodies) which constitutes the antitoxin is one of the serum proteins, and hence can not be completely separated from protein. The concentration of antitoxin without a similar concentration of protein is regarded by others as an indication that the antitoxin may be a body of non-protein nature.

Under these conditions any test which would conclusively decide whether an antitoxin is or is not identical with a serum protein would have both a practical and a theoretical interest. Accordingly, the fol-

[1] Reference is made by number (italic) to "Literature cited," pp. 494-495.

lowing test was decided upon because of its promising nature. If an antitoxin—for example, tetanus antitoxin—is a substance of nonprotein nature, it should be possible to prepare artificial digestion mixtures containing the antitoxic serum or derived globulin in such a manner that the protein would undergo digestion without loss of antitoxin. Appropriate chemical measurements would indicate the extent to which proteolysis has taken place, while inoculation experiments on guinea pigs would indicate whether there was any loss of antitoxic units. If, on the other hand, the antitoxin is a protein, and its power to immunologically neutralize the corresponding toxin is a function of the intact protein molecule, then the antitoxin would be destroyed in every case where the proteins had undergone cleavage, regardless of whether the cleavage was caused by a proteolytic enzym or other chemical agent. Due regard must, of course, be had for the possible destruction of the toxin by the chemical agents used.

However, these theoretical considerations were not the only ones that prompted the present investigation. For practical reasons numerous investigators studied the possibility of immunizing animals by administering the antitoxin by mouth. Some found that tetanus and diphtheria antitoxins were destroyed in the digestive tract; others found that the animals could be so immunized. The present work on the effect of digesting tetanus serum or derived globulin *in vitro* was expected to throw some light on the fate of immunity units administered *per os*.

WORK OF PREVIOUS INVESTIGATORS

Table I briefly summarizes the conclusions of the more important investigations on this subject. Of the two on tetanus serum by Carrière (*4*) and McClintock and King (*9*), it is doubtful whether either led to entirely correct conclusions. McClintock and King state (*p. 702*)—

> . . . we are now able to take series after series of guinea-pigs or rabbits, and by the oral administration of [tetanus, diphtheria] antitoxin save 100 per cent of the treated animals, when the antitoxin is administered before the toxin; while the untreated ones, receiving the same dose of poison, invariably die . . .

In their experiments with diphtheria and tetanus antitoxins (*p. 713*) administered to men by mouth they demonstrated the presence of the antitoxin in the blood of the men who had swallowed it a few days before the guinea-pig inoculation test. Their numerous positive immunizations by mouth led them to conclude that—

> . . . the diphtheria or tetanus antitoxin, which had been administered to the individual, had resisted digestion and had been absorbed in sufficient quantities to cause the protective antitoxin to be present in the blood of the individual . . .

These remarkable results by McClintock and King, although apparently obtained under carefully controlled conditions, have not carried conviction with them; they have not served as a starting point for

other investigations. Subsequent confirmation or contradiction of their work was not found, but practically all workers before them found that immunization by administration *per os* was not practicable. The immune bodies were destroyed or neutralized; most probably they were destroyed by the acid, the alkali, and the proteolytic enzyms of the digestive tract acting singly or in combination.

TABLE I.—*Summary of results of previous investigations on the action of proteolytic enzyms on antitoxins*

Investigation.	Digestive tract.	Pepsin-hydrochloric acid.	Hydrochloric acid.	Trypsin.
Belfanti and Carbone (*2*) diphtheria serum and diphtheria antitoxin.	.....	Destroyed.....	Destroyed by 0.1 per cent hydrochloric acid in a few hours.	Destroyed.
Carrière (*4*) tetanus serum	.....	Not affected in 24 hours.	.....	Destroyed appreciably in 24 hours. Alkali absent.
Dzierzgowski (*5*) diphtheria serum.	.....	Destroyed almost completely in 10 hours.	Destroyed almost completely by 0.5 per cent hydrochloric acid in 24 hours.	Not destroyed by pancreatic juice in 12 hours.
Nicolas and Arloing (*11*) diphtheria serum.	Destroyed.....	.....	.....	
Pick (*12*) diphtheria serum.	.....	.....	.....	Destroyed two-thirds by trypsin-alkali in 9 days.
McClintock and King (*9*) diphtheria serum and tetanus serum.	Not destroyed...	.....	.....	
Mellanby (*10*) diphtheria serum.	.....	Destroyed.....	Not affected by 0.25 per cent hydrochloric acid (time not stated).	Destroyed slowly in 9 days.

If it be assumed that McClintock and King actually immunized animals and man by *per os* administration and detected the antitoxin in the blood of individuals who had swallowed antitoxin, it does not follow that the antitoxin resisted digestion. It may have been absorbed from the digestive tract before the antitoxin had been destroyed.

Carrière (*4*) found that tetanus serum in pepsin-hydrochloric acid lost none of its immunizing power in 24 hours, a finding that is undoubtedly erroneous. He correctly found that trypsin appreciably destroyed the antitoxic properties in 24 hours.

On account of the many properties common to both diphtheria and tetanus serums, some of the results on diphtheria were included in Table I because they undoubtedly throw light upon one another. This table speaks for itself. It shows that in general the antibodies present in tetanus and diphtheria serums, or in globulin concentrates obtained from them, do not resist the action of the chemical agents present in the digestive tract. There are slight inconsistencies, but these do not invalidate the above generalization. Thus, Dzierzgowski (*5*) in 1899 concluded that diphtheria serum does not lose any of its immunizing power when mixed for 12 hours with active pancreatic juice (*p. 350*). The recently discovered antitrypsin (*7, p. 121*) present in normal and patho-

logical blood should have indicated to Dzierzgowski that this was too short a digestion period, for only three years later Pick (*12, p. 386*), using but 10 guinea pigs in his digestion experiments, correctly found that two-thirds of the antitoxin was destroyed by trypsin if given time enough—9 days. Mellanby's statement (*10, p. 405*) that 0.2 per cent hydrochloric acid has no destructive action on diphtheria antitoxin is at variance with that of numerous workers who found that it was destroyed.

On the practical side the net results of the numerous investigators, a few of whom are mentioned in Table I, indicate the impracticability of administering antitoxins by mouth. On the theoretical side they throw very little light on the problem of the nature of the antibodies themselves. The various investigators who attempted to separate a pure antitoxin from associated protein naturally sought a method by which the pure antitoxin would be obtained free or nearly free from protein, regardless of whether the proteins were destroyed during the purification. The converse did not occur to any of the workers whose publications have been studied—that is, to separate the antitoxin from the associated proteins, leaving the proteins entirely intact, but destroying the antitoxin.

For the purpose of proving that the antitoxin is not of protein nature, it is obviously immaterial whether one obtains pure antitoxin free from protein or the pure protein free from antitoxin so long as the separation actually is made. No one has succeeded in making the first separation; the writers have succeeded in making the second.

In the experiments here described the different antitoxic preparations were exposed to the action of trypsin-sodium-carbonate or to pepsin-hydrochloric-acid solutions for comparatively long periods of time, with suitable controls. (See Table III.) The extent to which digestion took place was then measured. The antitoxin remaining in the mixtures was estimated by inoculation tests on guinea pigs, carried out with slight modifications according to the methods described by Rosenau and Anderson (*13*). Anthrax serum was studied first, but as the results obtained were inconclusive, they are only briefly mentioned after the data on tetanus serum and tetanus antitoxin.

EXPERIMENTAL MATERIALS

Table II contains a brief description of the antitoxic preparations and proteolytic enzyms used.

GUINEA PIGS.—A total of 440 guinea pigs were used. These were obtained from the Bureau of Animal Industry's stock of 3,000 or more continually on hand at the Bureau's Experiment Station at Bethesda, Md. These animals have been bred for years from selected hardy stock and are undoubtedly very superior for experimental purposes. Only the obviously healthy animals, weighing between 320 and 380 gm. were selected at the station and forwarded to the Bureau's animal room 2 or

3 days before they were used for experimental purposes. Out of these 440, 250 died during the various observation periods. It is believed that without exception the deaths were all due to tetanus. No post-mortem examinations were made, it being evident that the deaths were due to tetanus either from the symptoms or from the time and number of deaths. In all cases the symptoms of tetanus were observed when recording the death as due to tetanus, except in experiment 21, in which 36 guinea pigs were inoculated between 3 and 4 p. m. None were "down" the next morning, but when next observed, at 9 p. m., 30 hours after inoculation, 23 were found dead without anyone's having observed any symptoms, while 12 controls were alive. Deaths were recorded as tetanus, as the time, grouping, etc., practically precluded other possibilities.

TABLE II.—*Preparations used in experiments*

Designation of preparation.	Where obtained.	Maker's statement of number of antitoxic units in 1 c. c. of the preparation.
Tetanus antitoxin No. 420F	Lederle antitoxin laboratories	300.
Tetanus serum No. 374	do	202.
Tetanus antitoxin X	A mixture, total volume 55 c. c. of the following makes: Slee; Parke, Davis & Co.; and Mulford.	Average 236.
Tetanus serum No. 268	Lederle antitoxin laboratories	200.
Pepsin 1	A 100-gm. sample of Parke, Davis & Co. 1:3000, purchased about May, 1912.	
Pepsin 2	A 200-gm. sample of pepsin puriss, Gruebler, imported about March, 1913.	
Trypsin 3	A 50-gm. sample of "trypsin Merck," purchased August, 1913.	
Trypsin 4	A 50-gm. sample of "trypsin Merck," purchased November, 1916.	
Tetanus toxin F4	Hygienic Laboratory, United States Public-Health Service.	Standard toxin test dose, 0.0007 gm.
Tetanus toxin F1	do	Do.

The death of a guinea pig within a few days after its receipt from the Bureau Experiment Station is such a rarity that it may be disregarded. But 1 occurred out of the 440 guinea pigs. By reason of the selection at the station such deaths are more likely to be due to mechanical injury than disease. The comments by Wahl (*14, p. 227*) on the well-known susceptibility of guinea pigs to intercurrent infections and sudden fatal epidemics, pneumonia, pleurisy, ascites, etc., while true of ordinary guinea pigs, certainly do not apply to the stock maintained by the Bureau.

PREPARATION OF DIGESTION MIXTURES.—These were prepared as indicated in Table III and used in sets of five for obvious reasons. Mixture A contained only the serum or antitoxin, and served as a standard for comparing the antitoxin contents of the different mixtures. Guinea pigs inoculated with quantities of mixture A equivalent to 0.1 unit of antitoxin or more always survived the test dose of toxin. Mixture B was to show the effect of sodium carbonate or hydrochloric acid on the antitoxin. Mixture C was to show the effect of trypsin or pepsin on the antitoxin. In mixture D the proteins present in the tetanus serum or antitoxin were digested by pepsin-hydrochloric-acid or trypsin-sodium-carbonate. In experiment 6 sodium hydroxid was used instead of sodium carbonate, with practically the same results. In mixture E the effect of the pepsin-hydrochloric-acid or trypsin-sodium-carbonate on the toxin was shown. The antitoxin and toxin were mixed one hour before inoculation; hence, it was necessary to be certain that the reagents at their final dilution under the conditions of the experiments do not destroy any of the toxin.

TABLE III.—*Composition of digestion mixtures and analytic data*

EXPERIMENTS 1 TO 3.1 (1 C. C. OF MIXTURE TO CONTAIN 60 UNITS)

Mixture.	Antitoxin or serum.		Water.	Acid or alkali.		Enzym.		Acid or alkali used to flocculate 2 c. c. of mixture.	Digestion period.	In 1 c. c. of antitoxin.	
	Number.	Quantity.		Kind.	Quantity.	Kind.	Quantity.			Total coagulable protein.	Amino nitrogen.[a]
		C. c.	*C. c.*		*Mgm.*		*Mgm.*	*C. c.*	*Days.*	*Mgm.*	*Mgm.*
A	Antitoxin 420 F	10	40.0	Sodium carbonate	0	Trypsin 4	0	0.05 *N*/50 acetic acid	0	59.0	0.53
B	do	10	40.0	do	250	do	0	.9 *N*/5 acetic acid	15	57.7	.....
C	do	10	40.0	do	0	do	200	.1 *N*/50 acetic acid	16	25.7	3.43(13)
D	do	10	40.0	do	250	do	200	.9 *N*/5 acetic acid	13	21.2	3.22(14)
					250						[b] 7.00
E	Anthrax globulin	2.5	47.5	do		do	200	.....	.....	.....	.....

EXPERIMENTS 4 TO 5.1 (1 C. C. OF MIXTURE TO CONTAIN 40 UNITS)

										In 1 c. c. of serum.	
A	Serum 374	10	40	Sodium carbonate	0	Trypsin 4	0	.....	0	.....	0.57
								0.7 *N*/50 acetic acid	6	74.2	.82
								.7 *N*/50 acetic acid	17	78.7	.70
B	do	10	40	do	250	do	0	.9 *N*/5 acetic acid	6	71.5	.70
								.9 *N*/5 acetic acid	17	76.2	.76
C	do	10	40	do	0	do	200	.1 *N*/50 acetic acid	6	32.5	5.60
								.1 *N*/50 acetic acid	17	14.5	6.62
D	do	10	40	do	250	do	200	.9 *N*/5 acetic acid	6	31.5	3.92
								.9 *N*/5 acetic acid	17	16.5	4.29
											[b] 9.92
E	Anthrax serum 160	10	40	do	250	do	200	.....	.....	.....	.....

[a] Figures in parentheses are time (days) of digestion.

[b] Total amino nitrogen by hydrochloric acid hydrolysis. See p. 482.

TABLE III.—*Composition of digestion mixtures and analytic data*—Continued

Mixture.	Antitoxin or serum.		Water.	Acid or alkali.		Enzym.		Acid or alkali used to flocculate 2 c. c. of mixture.	Digestion period.	In 1 c. c. of antitoxin.	
	Number.	Quantity.		Kind.	Quantity.	Kind.	Quantity.			Total coagulable protein.	Amino nitrogen.[a]
		C. c.	*C. c.*		*C. c.*		*Mgm.*	*C. c.*	*Days.*	*Mgm.*	*Mgm.*
EXPERIMENTS 6 AND 6.1 (1 C. C. OF MIXTURE TO CONTAIN 47 UNITS)											
A	Antitoxin X	10	40	Sodium-hydroxid solution *N/172*.	0	Trypsin 3		0.6 *N/50* acetic acid	8	[b] 110.9	1.73
B	do	10		do	40	do		.1 *N/50* acetic acid	8	25.7	11.51
C	do	10	40	do	0	do	200	.6 *N/50* acetic acid	8	110.9	1.69
D	do	10		do	40	do	200	.1 *N/50* acetic acid	8	25.7	11.33
											[c] 13.73
E	Anthrax serum 160	10		do	40	do	200				
EXPERIMENT 20 (1 C. C. OF MIXTURE TO CONTAIN 60 UNITS)											
A	Antitoxin 420 F	10	40	2 per cent hydrochloric acid.	0	Pepsin 1		0.1 *N/50* acetic acid	12	61.6	
									14	63.5	
B	do	10	35	do	5	do		.55 *N/5* sodium hydroxid.	12	62.0	
									14	62.0	
C	do	10	40	do	0	do	100	.10 *N/50* acetic acid	12	[b] 49.0	
								.00	14	57.2	
D	do	10	35	do	5	do	100	.55 *N/5* sodium hydroxid.	12	1.5	
									14	2.3	
E	Normal horse serum	10	35	do	5	do	100				
EXPERIMENT 21 (1 C. C. OF MIXTURE TO CONTAIN 60 UNITS)											
A	Antitoxin 420 F	10	40	2 per cent hydrochloric acid.	0	Pepsin 1		0.1 *N/50* acetic acid	3	65.1	0.62(8)
B	do	10	35	do	5	do		.55 *N/5* sodium hydroxid.	3	59.7	0.67(8)
C	do	10	40	do	0	do	100	.00	3	58.5	0.76(8)
D	do	10	35	do	5	do	100	.55 *N/5* sodium hydroxid.	3	3.5	1.82(8)
											[c] 7.06
E	Normal horse serum	10	35	do	5	do	100				

EXPERIMENT 22 (1 C. C. OF MIXTURE TO CONTAIN 40 UNITS)

										In 1 c. c. of serum.	
A	Serum 268	10	40	2 per cent hydrochloric acid.	0	Pepsin 2		0.6 N/50 acetic acid	4	97.5	0.56(5)
B	do	10	35	do	5	do		.5 N/5 sodium hydroxid.	4	95.2	1.01(5)
C	do	10	40	do	0	do	100	.3 N/50 acetic acid	4	92.0	1.12(5)
D	do	10	35	do	5	do	100	.4 N/5 sodium hydroxid.	4	4.7	2.73(5)
											c 11.78
E	Normal horse serum	10	35	do	5	do	100				

a Figures in parenthesis are time (days) of digestion. b See p. 481. c Total amino nitrogen was obtained by hydrochloric-acid hydrolysis. See p. 482.

The mixtures were contained in wide-mouthed 100-c. c. bottles, and were closed with rubber stoppers tied firmly in place. The mixtures were always saturated with chloroform and kept in an incubator room at 37.5° C. Culture tests made from time to time showed that the mixtures were sterile.

ANALYTIC DATA

At suitable intervals, usually the day before or after the guinea pigs were inoculated, chemical analyses of the mixtures were made for the purpose of ascertaining the extent of protein digestion. The determinations made were coagulable protein and amino nitrogen. Inasmuch as details on the preparation and analyses of digestion mixtures have been published elsewhere (*3*), they will be omitted here.

TOTAL COAGULABLE PROTEIN.—The protein was precipitated, coagulated, centrifuged, dried, and weighed in centrifuge tubes (heavy glass bacteriological test tubes), the outer dimensions of which were: Length 95 mm., diameter 17 mm. Eight such tubes, cleaned, dried, and weighed to 0.1 mgm., were used at a time, two for each of the mixtures A, B, C, and D. No analyses were made of mixture E. Into each tube 7 c. c. of water and 2 c. c. of digestion mixture or 9 c. c. of water and 1 c. c. of serum or antitoxin were measured, with Ostwald pipettes of 1 and 2 c. c. delivery for the latter. Each tube was warmed over a Bunsen burner, and sufficient dilute acetic acid or sodium-hydroxid solution was added to precipitate completely the protein, after which the contents of the tube were brought almost to a boil. The tubes were then centrifuged for 20 to 25 minutes at about 2,400 revolutions per minute. When the flocculation had been properly performed, the coagulated protein packed firmly to the bottom, leaving a water-clear supernatant fluid which may be poured off without appreciable loss of precipitate, even if the tube is inverted. The tubes were then immersed in a sulphuric-acid-potassium-dichromate cleaning mixture, rinsed, and dried on the outside to make certain that there was no adherent dirt. The tubes containing the moist precipitates were dried to constant weight at room temperature in a sulphuric-acid desiccator evacuated to about 10 mm. of mercury. The drying was not difficult; usually a single drying of 24 hours was sufficient, provided the acid was renewed very frequently—that is, after two dryings. In every case the drying was continued until the loss in weight of a precipitate did not exceed a few tenths of a milligram. The difference between the weight of the tube empty and the weight with the precipitate gave the weight of coagulable protein.

These tubes, which had been numbered with hydrofluoric acid, after being used were cleaned with hot dichromate mixture, washed, dried in the hot-air oven, placed in the desiccator, and weighed, ready for the next determinations. This method for total coagulable protein gives

quick and accurate results. Thus in experiment 6 the following were obtained with 2 c. c. of the mixtures containing 0.4 c. c. of antitoxin X: Mixture A, 43.3, 44.8 mgm.; mixture C, 43.8, 44, 44.8, 45.4 mgm.; mixture B, 9.8, 10.2, 10.2, 10.5 mgm.; mixture D, 10.4, 10.5 mgm. In this experiment an error was made by the addition of trypsin to mixture B instead of C, resulting in two pairs of identical mixtures, one of which contained only the antitoxin, the other trypsin-sodium-hydroxid in addition.

The most difficult step in the determination of coagulable protein is the complete flocculation of the protein. The above-mentioned close duplicates probably will not be obtained by the inexperienced or those who do not realize the effect of small quantities of acid or alkali in this determination. This point is well illustrated by the following data obtained in experiment 20 (see Table III): To 2 c. c. of mixture C containing 0.4 c. c. of antitoxin 420F., 7 c. c. of water were added, and the protein was flocculated with 0.10 c. c. *N*/50 acetic acid. The total coagulable protein obtained was 19.4 and 20 mgm. The average of these two, calculated to 1 c. c. of antitoxin, gave 49 mgm., the result given in Table III. Two days later the determinations were repeated, but neither acid nor alkali was used for flocculation. Obtained 22.5 and 23.3 mgm., which, calculated as before, gave 57.2 mgm. total coagulable protein per 1 c. c. of antitoxin. So small a difference as 0.1 c. c. of *N*/50 acetic acid made an appreciable difference in the result—that is, 8 mgm.

It will be noticed that the figure for mixture C, 57.2 mgm., is appreciably lower than that for mixture A, 63.5 mgm., as if 9 per cent of the protein had been digested past the coagulable stage. Mixture C contained pepsin only, and this in all probability exerted no digestive action in the neutral solution. In general it was found that the addition of pepsin or trypsin to a mixture lowered the amount of protein coagulated when the coagulation was attempted immediately after the addition. Thus, in experiment 3, eight 2-c. c. portions of mixture B were pipetted into as many centrifuge tubes. Four were coagulated in the usual way with 0.9 c. c. of *N*/5 acetic acid. There were obtained 22.1, 22.2, 24.0, and 24.2 mgm. of coagulable protein. To the other four tubes weighed portions of trypsin 4 were added, varying from 27 to 72 mgm., and the coagulation carried out as before without allowing any time for the trypsin to act proteolytically. The coagulable protein obtained amounted to 19.0, 19.5, 20.2, and 20.3 mgm. Plainly the trypsin lowered the amount of coagulable protein by 3.4 mgm. A correction was not made, because 2 c. c. of the digestion mixture contained but 8 mgm. of trypsin, and the correction probably varied at different stages of digestion. This effect is regarded as the probable explanation of the apparent digestion in mixtures C, experiments 20 to 22.

To calculate the amount of coagulable protein digested past the coagulable stage, in experiment 22 for example, from the analytic data

in Table III, 1 c. c. of serum 268 in mixture A contained 97.5 mgm. of coagulable protein four days after the mixture had been prepared. In mixture D digestion was going on, and 1 c. c. of serum in this mixture contained but 4.7 mgm. of coagulable protein. The other 92.8 mgm., 95 per cent of the total, had been transformed into proteoses and peptones which do not coagulate on heating. This figure, 95 per cent, together with others similarly calculated, is to be found in Table VII.

Amino nitrogen.—Although the determination of coagulable protein digested past the coagulable stage, as described, gives valuable information regarding the extent of digestion, further information was obtained by determining the amino nitrogen liberated at the same time. Van Slyke's method and apparatus were used as described in a previous publication by the senior writer (*3, p. 696*). For analytic purposes ammonia was removed in the following manner: At the close of the digestion period 10-c. c. portions of the various mixtures were transferred to porcelain crucibles, to each of which 0.5 c. c. of concentrated hydrochloric acid was added to destroy the trypsin at the desired time. These were allowed to stand for one hour and then made faintly but distinctly alkaline to litmus strips by the addition of about 0.3 gm. of sodium carbonate. The crucibles were then placed in a vacuum desiccator provided with a shallow dish containing some *N/5* sulphuric acid. No attempt was made to estimate the ammonia. That the removal was complete was shown by numerous blanks with pure ammonium sulphate in this and in the following procedure. The next day the contents of the crucibles were used for the amino-nitrogen determinations; 5 or 10 c. c. of digestion mixture were used for one determination, according to the amount of amino nitrogen present. The results are given in Table III, last column, and have been corrected for amino nitrogen in the trypsin and pepsin. The amounts found in mixture A represent the preformed amino nitrogen present in the serum or antitoxin, and were subtracted from the amounts found in the other mixtures, when calculating the amount liberated by the digestion.

For total amino-nitrogen determination, 10 c. c. of serum or antitoxin were boiled with 10 c. c. of concentrated hydrochloric acid for 24 hours under a reflux condenser. The mixture was evaporated to dryness to expel hydrochloric acid, made alkaline with sodium-carbonate solution, keeping the total volume small, and set aside in an incubator room for 24 to 48 hours for the ammonia to escape. The mixtures usually evaporated almost to dryness. They were taken up with water and 0.5 c. c. concentrated hydrochloric acid, transferred to a 50-c. c. volumetric flask and diluted to the mark. For a single determination in the amino-nitrogen apparatus, 5 or 10 c. c. of the unfiltered solution were used, containing 1 or 2 c. c., respectively, of hydrolyzed serum or antitoxin.

Pepsin-acid mixtures were introduced directly into the Van Slyke apparatus without removal of ammonia, as the amounts were too small

to have an appreciable influence. The determinations of coagulable protein and amino nitrogen in trypsin mixtures were made within a day or two of one another, but in the peptic mixtures a longer interval was regarded as permissible on account of the much slower liberation of amino nitrogen. The results are tabulated below. The amounts of amino nitrogen per gram of coagulable protein are practically the same in tetanus serum, anthrax serum, and ordinary beef and veal muscle tissue.

Milligrams of amino nitrogen per gram of coagulable protein

Tetanus antitoxin, 420 F	117.4
Tetanus serum, 374	133.2
Tetanus serum, 268	120.8
Average of 3	123.8
Tetanus antitoxin X[a]	123.8
Anthrax serum, 48	110.3
Anthrax serum 48.3	120.2
Average of 5	120.4
Normal beef or veal muscle tissue (*Berg, 3, p. 680*). On the basis of 16 per cent of nitrogen in protein	116.8
Above tissue freed from extractives	120.0

Banzhaf, Sugiura, and Falk (*1*) made analyses and determined the nitrogen distribution of a number of antiserums, but found no marked differences in the compositions of the proteins in normal serum, tetanus, and diphtheria globulins, etc. The above figures are in accord with the findings of these investigators. All indicate that the normal serum proteins, as they pass over into immune serum proteins, do not undergo chemical changes that are detectable by present methods. The same is probably true of tissue proteins.

To calculate the percentage of the total amino nitrogen liberated by digestion, in experiment 22, for example, from the analytic data in Table III, 1 c. c. of serum 268 in mixture D contained 2.73 mgm. of amino nitrogen after 5 days' digestion; at the same time a similar quantity of serum in mixture A, in which no digestion was taking place, contained 0.56 mgm. Therefore, in mixture D, $2.73-0.56=2.17$ mgm. of amino nitrogen were liberated by the digestion for each cubic centimeter of serum. If all of the amino nitrogen were thus liberated, 11.78 mgm. would be present; hence, $2.17/11.78=18$ per cent of the total was liberated by digestion. This figure, 18 per cent, together with other figures similarly calculated, is to be found in Table VII.

INOCULATION TESTS

The amounts of antitoxin remaining in the various mixtures were determined, after suitable digestion periods, according to the method described by Rosenau and Anderson (*13*).

[a] For antitoxin X, a lack of material for analysis necessitated the assumption of the above average.

An example of a test.—Experiment 5. On May 4, 1917, mixtures containing tetanus serum 374 were prepared as detailed in Table III. The same mixtures were used in experiments 4, 5, and 5.1. Chemical data were obtained 6 and 17 days after digestion began. On the day of the inoculation, 18 days after digestion began, a 0.5-c. c. portion of each mixture was diluted to 20 c. c. with salt solution, resulting in five dilute antitoxin solutions, all of which contained 1 unit per cubic centimeter if none of the antitoxin had been destroyed. Of course, no antitetanus units were present at any time in mixture E. Measured quantities of these diluted mixtures were then mixed with salt solution and tetanus-toxin solution as detailed in Table IV, which is a typical one. As three guinea pigs were used on each dose, 3 or 4 doses were prepared; later but 2 guinea pigs on each dose were used, beginning with experiment 6.

Table IV.—*Composition of the doses injected (experiment 5)*

[0.50 c. c. of digestion mixture was diluted to 20.0 c. c. with physiological salt solution; 1 c. c. of diluted mixture contained 1 unit of antitoxin if none had been destroyed]

Mixture.	Diluted mixture.	Salt solution.	Toxin solution.	Injected into 1 guinea pig.	Dose originally contained.
	C. c.	*C. c.*	*C. c.*	*C. c.*	*Unit.*
Mixture A	0.07	0.93	1.00	2.00	0.07
	.10	.90	1.00	2.00	.10
	.20	.80	1.00	2.00	.20
Mixtures B, C, D	.10	.90	1.00	2.00	.10
	.20	.80	1.00	2.00	.20
	.40	.60	1.00	2.00	.40
	.80	.20	1.00	2.00	.80
Mixture E	.80	.20	[a] 1.00	2.00	.00

[a] In all experiments after this, but 0.02 c. c. of toxin solution was mixed in the dose of mixture E for 1 guinea pig.

It was possible that the trypsin and sodium carbonate or pepsin and hydrochloric acid at their final dilutions might destroy 50 or 60 MLD (minimal lethal dose) out of the 100 in the test dose, and still the mixture E guinea pigs would die within the standard time of 96 hours. But by giving this group of pigs but 2 MLD instead of 100, any destructive action on the toxin would have manifested itself. Out of 12 such guinea pigs in 6 experiments, 9 died within 96 hours, 1 within 120 hours, 1 within 7 days, and 1 lived, indicating that there was no significant destruction of toxin.

Two samples of tetanus toxin, labeled "F1" and "F4," were obtained from the Hygienic Laboratory of the United States Public Health Service. The test dose (100 MLD) was the same for both, 0.0007 gm. The toxins were kept in a Hempel sulphuric-acid desiccator evacuated to 3 mm. of mercury, in a refrigerator room at 1° C, in the dark.

The toxin and antitoxin were not mixed in separate syringes as practiced by Rosenau and Anderson; instead they were mixed in weighing

bottles (clean and sterile) having a capacity of about 50 c. c., height 50 mm., diameter 40 mm. These serve as excellent containers. Thus when two guinea pigs were to be injected with similar doses, three doses were prepared by mixing 3 c. c. of toxin solution and 3 c. c. of diluted antitoxin mixture. After standing for one hour in diffuse sunlight at room temperature the doses were injected, 2 c. c. into one guinea pig.

In the following tables (V–VI) the results of two typical inoculation tests are detailed. Although the tests closed at the expiration of 96 hours after inoculation, the guinea pigs were always kept under observation for an additional 96 hours. The time of death is recorded in the tables; when no such time is recorded, it means that the animals receiving that particular dose were alive seven or eight days after inoculation. Guinea pigs that died during the night, between 11 p. m. and 8 a. m., were recorded as having died at 8 a. m. Otherwise, dead guinea pigs seldom remained in their cages for more than one hour.

TABLE V.—*Destruction of antitoxin by trypsin and sodium carbonate (experiment 5)*

[Tetanus serum 374. Toxin F4. Digestion period, 18 days on day of injection. Total of 48 guinea pigs, in 16 groups of 3 on each dose]

Mixture A (serum only).		Mixture B (sodium carbonate).		Mixture C (trypsin).		Mixture D (sodium carbonate and trypsin).		Mixture E (sodium carbonate and trypsin).	
Unit.	Died.	Unit.	Died.	Unit.	Died.	Unit.	Died.	Unit.	Died.
	Hours.		*Hours.*		*Hours.*		*Hours.*		*Hours.*
0.07	114 138 192	0.10	25 31 42	0.10	31 42 42	0.10	31 42 42	0.00	20 25 31
.10	.....	.20	31 42 42	.20	66 66 66	.20	43 55 55		
.20	.....	.40	55 66 66	.40	.....	.40	114 138 144		
		.80	.....	.80	.....	.80	.....		

ANTITOXIN DESTROYED (PER CENT)				
0	91	82	[a] 82	.....

COAGULABLE PROTEIN DIGESTED PAST COAGULABLE STAGE (PER CENT)				
0	0	80	78	.....

AMINO NITROGEN LIBERATED BY DIGESTION (PER CENT)				
0	0	57	35	.....

[a] For the method of calculating these percentages see p. 481, 483, 487.

TABLE VI.—*Destruction of antitoxin by pepsin and hydrochloric acid (experiment 21)*

[Tetanus antitoxin 420 F. Toxin F1. Digestion period, 4 days on the day of injection. Total of 36 guinea pigs in 18 groups of 2 on each dose]

Mixture A (antitoxin only).		Mixture B (hydrochloric acid).		Mixture C (pepsin).		Mixture D (pepsin and hydrochloric acid).		Mixture E (pepsin and hydrochloric acid).	
Unit.	Died.	Unit.	Died.	Unit.	Died.	Unit.	Died.	Unit.	Died.
	Hours.		*Hours.*		*Hours.*		*Hours.*		*Hours.*
0.07	46	0.10	30	0.07	49	0.10	30	0.00	66
	66		42		66		30		90
.10	114	.20	30	.10	94	.20	30	b	.....
	114		30		114		30		
		.30	30	.15	.....	.30	30		
			30				30		
		.40	30			.40	30		
			30				30		
		.60	30			.60	30		
			30				30		
		.80	30			.80	30		
			30				30		
ANTITOXIN DESTROYED (PER CENT)									
0		Over 87		0		Over 87		.....	
COAGULABLE PROTEIN DIGESTED PAST COAGULABLE STAGE (PER CENT)									
0		8		a 10		95		.....	
AMINO NITROGEN LIBERATED BY DIGESTION (PER CENT)									
0		0		0		17		.....	

a See p. 481. b Only 2 MLD in 1 dose of E.

INTERPRETATION OF ANALYTIC DATA AND INOCULATION TESTS

The term "unit" or "immunity unit" is used in this work in the sense defined by Rosenau and Anderson (*13, p. 6*) as follows:

> The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public-Health and Marine-Hospital Service.

At the time this definition was made (1907) the toxin was the standard and the antitoxin a derived or secondary standard. According to this definition, it was immaterial whether the guinea pig died or lived after the 96 hours. At the time of this writing (1917) the Hygienic

Laboratory had slightly changed the definition, as follows (from a typewritten statement):

> The test dose of the tetanus toxin F is 0.0007 gram. This should kill a 350-gram guinea pig in about 96 hours, when mixed with one-tenth unit of antitoxin. The test dose contains approximately 100 minimal lethal doses.

According to this definition, the antitoxin is now the standard from which the toxin is derived, and it is not immaterial whether the guinea pig is alive or dead after 96 hours—the 0.1 unit as later defined specifies that the guinea pig shall not live more than 96 hours if the dose contain but 0.1 unit. Either definition used consistently would undoubtedly lead to the same conclusions in this work, since these are based upon comparisons made under similar conditions rather than upon absolute values. The older definition was used, as it seemed to be more convenient. All doses of antitoxin which protected a guinea pig for 96 hours were regarded as containing at least 0.1 unit. There was generally little need for fine distinctions, as few of the guinea pigs died just before the 96-hour limit. With few exceptions these guinea pigs were regarded as having received less than 0.1 antitoxic unit, according to the older definition. In the following statement the number of guinea pigs that died before the time indicated is shown. All of them had received the test dose of toxin plus varying amounts of antitoxin. Twelve guinea pigs that received only 2 MLD are not included.

Time after inoculation:	Number of guinea pigs dead.
20 to 24 hours	24
25 to 48 hours	131
49 to 72 hours	36
	[a] 191
73 to 96 hours	19
97 to 120 hours	17
121 to 144 hours	9
145 to 168 hours	3
169 to 192 hours	1
Total	240

Calculation of the percentage of the total antitoxin destroyed.—It will be noticed in Table V that the three guinea pigs (and three others) that received a quantity of serum 374 containing 0.07 unit were protected for more than 96 hours against the test dose of toxin. Consequently the quantity of serum calculated to contain 0.07 unit, on the basis of the manufacturer's statement that the serum contained 202 units per cubic centimeter (see Table II) actually contained at least 0.1 unit, the manufacturer apparently having liberally understated the

[a] Dead in 72 hours.

potency, partly to allow for deterioration with time and partly in the desire to furnish promptly the product when requested. In a similar way, antitoxin X and serum 268 were found to have potencies about 50 per cent higher than that stated. The other preparation used, antitoxin 420F, stated to contain 300 units per cubic centimeter, protected guinea pigs for 96 hours in 0.1 unit doses, but not in 0.07 unit doses (see Table VI). This preparation had received a final and correct standardization before shipment. Therefore all the doses in Table V should be multiplied by 10/7. In mixture B the only guinea pigs that lived more than 96 hours were those that had received a quantity of serum calculated to contain 0.80 unit if none of the potency had been destroyed. The results with mixture A show that this dose contained originally, at least, 0.80 times 10/7, or 1.14 units. At the time of injection this dose (B) contained at least 0.1 unit, because the guinea pigs were protected for over 96 hours. Hence, at most, the destruction was $\frac{1.14-0.1}{1.14}$, or 91 per cent. The guinea pigs that received the next lower dose—that is, 0.40 times 10/7, or 0.57 units—died in less than 96 hours; therefore this dose contained less than 0.1 unit, or, over 0.47 out of 0.57 unit was destroyed (82 per cent). Consequently in this experiment over 82 but less than 91 per cent of the antitoxin in mixture B was destroyed. The higher figure was used throughout.

In Table VI the results with mixture A show that the doses actually contained the number of units stated in the table. In mixture D, for example, the 0.8-unit dose contained less than 0.1 unit, or the destruction was more than $\frac{0.8-0.1}{0.8}$, or 87 per cent. It is believed that the figures for percentage of antitoxin destroyed may be incorrect by a few points, but this is not of importance.

Comparison of antitoxin destroyed with protein digested.—Twelve inoculation tests were made, but all are not recorded here in detail, because Tables V and VI are thoroughly typical of the others. A discussion of the results of experiments 5 and 21 will apply to the others, because of their general concordance. With the exception of experiments 1 and 2, which were preliminary, the remaining 10 experiments, in which 360 guinea pigs were used, are summarized in Table VII.

TABLE VII.—*Comparison of antitoxin destroyed with protein digested*

EXPERIMENTS 3–6.1 : TRYPTIC DIGESTION

Experiment No.	Antitoxin No.	Toxin No.	Digestion period on day of inoculation.	Mixture.	Antitoxin destroyed.	Coagulable protein digested past coagulable stage.	Amino nitrogen liberated by digestion.
			Days.		*Per cent.*	*Per cent.*	*Per cent.*
3	420 F.	F4	13	[a] A	0	0	0
				B	87	4	.....
				C	75	57	40
				D	87	65	37
3.1	420 F.	F4	119	A	0	.....	.....
				B	over 95	.....	.....
				C	75–87	.....	.....
				D	over 95	.....	.....
4	Serum 374.	F4	8	A	0	0	0
				B	65	0	0
				C	65	56	53
				D	65	58	31
5	Serum 374.	F4	18	A	0	0	0
				B	91	0	0
				C	82	80	57
				D	82	78	35
5.1	Serum 374.	F4	76	A	0	.....	.....
				B	96	.....	.....
				C	82–91	.....	.....
				D	82–91	.....	.....
6	Antitoxin X.	F1	9	A	0	0	0
				B	82	77	[b] 71
				C	0	0	0
				D	82	77	70
6.1	Antitoxin X.	F4	45	A	0	.....	.....
				B	82–93	.....	.....
				C	0	.....	.....
				D	82–93	.....	.....

EXPERIMENTS 20–22 : PEPTIC DIGESTION

20	420F.	F4	11	[c] A	0	0	.....
				B	over 87	0	.....
				C	0	[d] 9	.....
				D	over 87	96	.....
21	420F.	F1	4	A	0	0	0
				B	over 87	8	0
				C	0	[d] 10	0
				D	over 87	95	17
22	Serum 268.	F1	3	A	0	0	0
				B	over 96	2	4
				C	0	6	5
				D	over 96	95	18

[a] In these mixtures A contained only antitoxin; in addition to this the others contained: B, sodium carbonate; C, trypsin; D, sodium carbonate and trypsin.
[b] See page 481.
[c] In these mixtures A contained only antitoxin; in addition to this the others contained: B, hydrochloric acid; C, pepsin; D, pepsin and hydrochloric acid.
[d] See page 481.

OBSERVATIONS ON RESULTS OF EXPERIMENTS 5 AND 21

The following observations on the results of experiment 5 (Table V) are true of the other experiments in which trypsin was used.

MIXTURE A.—This mixture contained only the serum and was always used as the standard with which the other mixtures were compared. Such a mixture undergoes very little change on standing and may be regarded as having a constant chemical composition and potency for periods such as those indicated in Table VII. All the mixtures were prepared under conditions assuring sterility. Bacterial action in the mixtures was prevented by chloroform. The results of mixture A served to standardize the technic of the experiments. For example, if the toxin used had undergone a loss in toxicity, it would have been apparent from results with this mixture. One test was made with a standard tetanus antitoxin, T17, furnished by the Hygienic Laboratory. Twelve guinea pigs were used, two on each dose, each of which received 0.1 unit of the standard antitoxin plus the following doses of toxin: Toxin F4; 0.00065, 0.0007, and 0.00075 gm. Toxin F1: 0.00065, 0.0007, and 0.00075 gm. The results indicated that at the time of this test (experiment 4, May 18, 1917), the standard antitoxin and toxin were of standard strength, and the experimental technic used was correct.

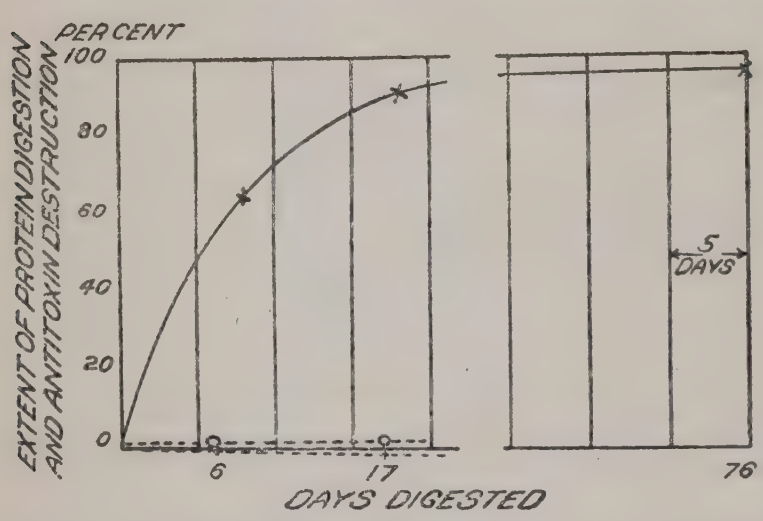

FIG. 1.—The destruction of tetanus antitoxin (—— × —) in 0.5 per cent sodium-carbonate solution without any change in total coagulable protein (– – ○ – –) or amino nitrogen (— + ——). Mixture B, experiments 4–5.1.

MIXTURE B.—This contained serum in 0.5 per cent sodium-carbonate solution. (The mixture B for experiment 6–6.1 contained antitoxin X in a final concentration of *N/215* sodium hydroxid instead of the carbonate. The two solutions are equally alkaline.) In this mixture the antitoxin was slowly but completely destroyed by the sodium carbonate (or hydroxid). After 18 days' contact between the antitoxin and alkali (experiment 5) 91 per cent of the antitoxic units had disappeared; but during this time the data for total coagulable protein and amino nitrogen indicate that the protein contents of this mixture underwent no change. It would appear that in this way the antitoxin may be separated from the associated protein, but during the separation the antitoxic units are destroyed, leaving the proteins intact. This effect was not recorded by

any of the previous investigators mentioned on page 473; it probably was not looked for.

The figures in Table II for mixture B, experiment 5, are shown graphically in figure 1. From such data, if none other were available, the conclusion would be justified that the antibodies were separate from the proteins, so far as one may be almost totally destroyed without any detectable difference in the other.

MIXTURE C.—This mixture contained serum and trypsin. The original object was to make certain that the trypsin was immunologically neutral under the experimental conditions. The mixture was faintly amphoteric to litmus-paper strips. In the experiment 3 mixture it was faintly acid. The digestion in mixture C was as rapid as in mixture D, which contained 0.5 per cent of sodium carbonate and trypsin. In 18 days, in mixture C, 80 per cent of the coagulable protein was digested past the coagulable stage and 57 per cent of the total amino nitrogen was liberated. During this same time, 82 per cent of the antitoxin units were destroyed. The experimental data are graphically represented in figure 2. Because the rate of protein splitting is practically the same as the rate of antitoxin destruction in such a mixture, the supposition that the antitoxin and the protein are identical substances would be a reasonable one were it not for the fact that the data with mixture B show that the antitoxin may be destroyed without protein splitting.

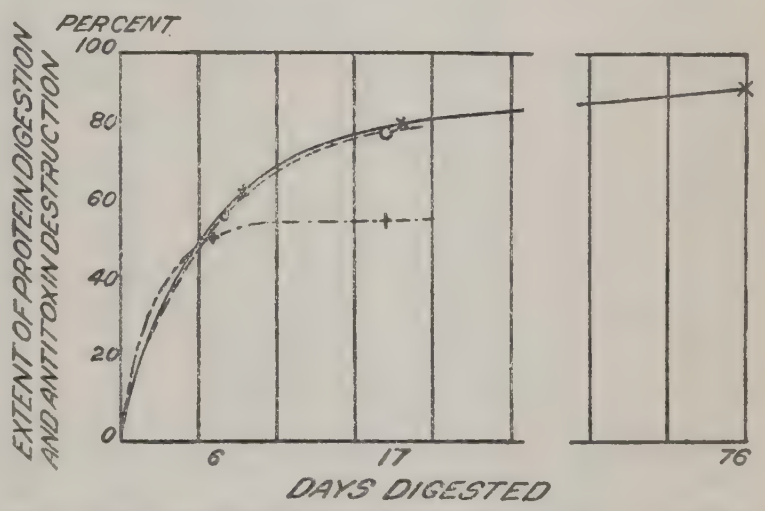

FIG. 2.—The destruction of tetanus antitoxin (——X——) by trypsin in solution amphoteric to litmus strips; the digestion of coagulable protein past the coagulable stage (— —○— —); the liberation of free amino nitrogen (—— ——+—— ——). Mixture C, experiments 4–5.1.

MIXTURE D.—The results were practically the same as with mixture C.

MIXTURE E.—This contained trypsin and sodium carbonate in the same quantities as mixture D, but no tetanus serum. Instead anthrax serum was used; in other E mixtures normal horse serum was used. This mixture obviously contained no tetanus antitoxic units, and was used for the purpose of ascertaining with certainty that the tetanus toxin was not destroyed by the largest amounts of trypsin and sodium carbonate in doses of other mixtures. (See page 484.) The highest final concentration of sodium carbonate in the toxin-antitoxin mixture injected was 0.008 per cent.

The following observations on the results of experiment 21 (Table VI) are true of the other experiments in which pepsin was used.

MIXTURE A.—This contained only antitoxin 420 F and was used as a standard with which the other mixtures were compared.

MIXTURE B.—This contained the antitoxin in 0.2 per cent hydrochloric-acid solution. From the data in Tables III, VI, and VII it is apparent that the acid destroyed all or very nearly all of the antitoxin in four days, during which time the proteins underwent practically no change. There was a slightly smaller amount of coagulable protein in mixture B than in mixture A, but in the other two B mixtures (experiments 20, 22) the differences were insignificant. The figures for amino nitrogen likewise indicate that while over 87 per cent of the antitoxin was destroyed, the changes in the proteins, as measured chemically, were so slight as to justify the conclusion that under these particular experimental conditions the destruction of the antitoxin involves no protein breakdown. The destruction of over 96 per cent of tetanus antitoxin in mixture B, experiment 22, without any significant change in the amounts of total coagulable protein or amino nitrogen are graphically represented in figure 3. This effect was not recorded by any of the previous investigators mentioned on page 473; it probably was not looked for. This affords a second method of separating tetanus antitoxin from associated protein; but the antitoxin is destroyed, leaving the antitoxin-free proteins intact.

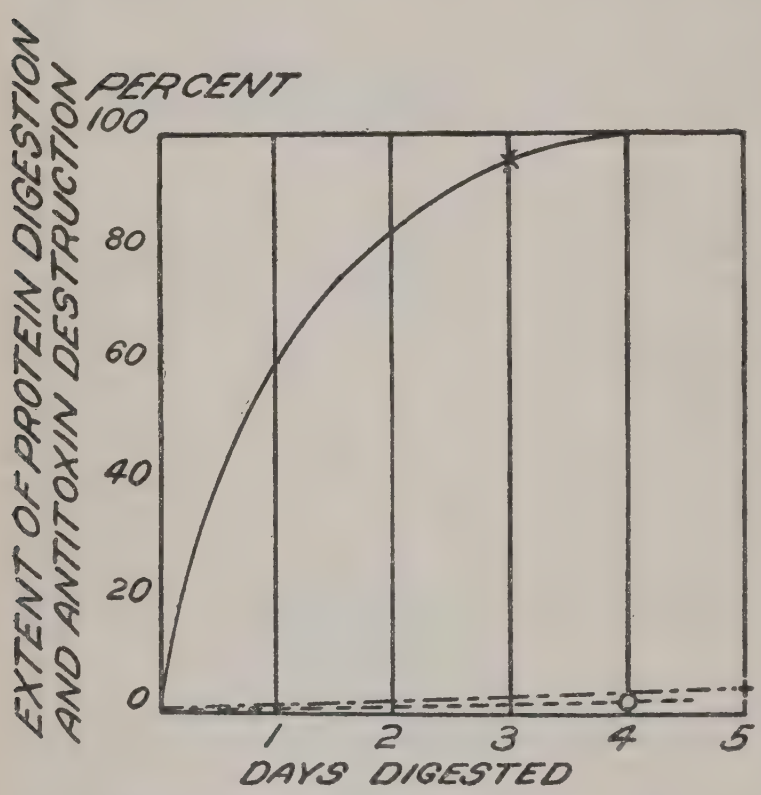

FIG. 3.—The destruction of tetanus antitoxin (——X——) by 0.2 per cent hydrochloric acid without any significant change in the amounts of total coagulable protein (— —O— —) or free amino nitrogen (— —+— —). Mixture B, experiment 22.

MIXTURE C.—There were no changes in this mixture containing antitoxin and pepsin—that is, there was neither antitoxin destruction nor protein digestion. The lower figures for total coagulable protein, as compared with mixture A, do not indicate a slight proteolysis, but rather a difficulty of obtaining a complete precipitation of the protein in the

presence of the proteoses, peptones, etc., which make up the bulk of the trypsin and pepsin preparations used, as explained on page 481.

MIXTURE D.—The destruction of antitoxin by pepsin-hydrochloric acid in this mixture was obviously due to the hydrochloric acid, as the results for mixture B indicate. Furthermore, these indicate that the proteolysis in mixture D can not with certainty be regarded as the immediate cause of the antitoxin destruction.

Figure 4 shows the simultaneous antitoxin destruction and protein splitting in mixture D, experiment 22.

Carrière's (*4*) finding that pepsin-hydrochloric acid does not affect tetanus antitoxin is probably incorrect.

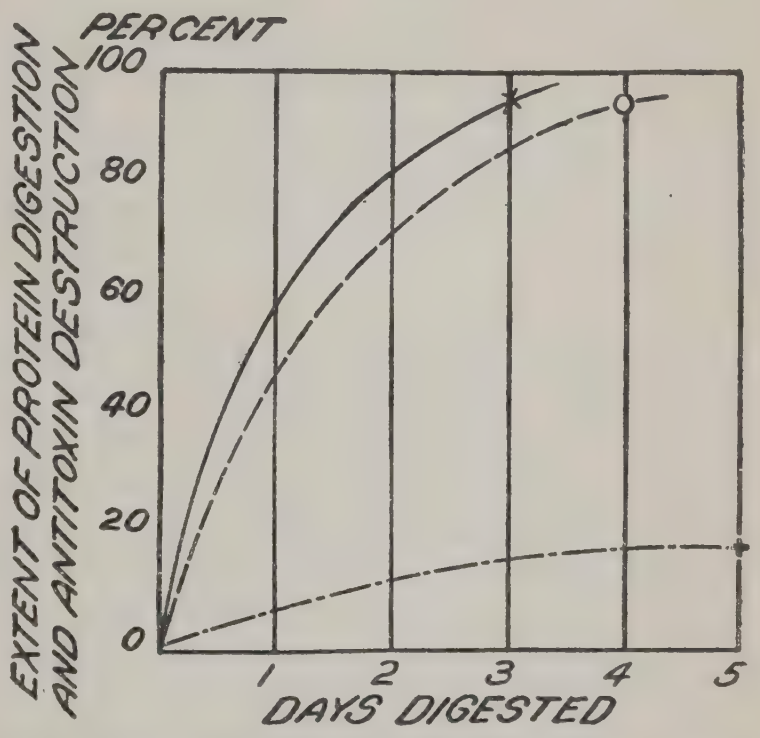

FIG. 4.—The destruction of tetanus antitoxin (——X——) by pepsin-hydrochloric acid; the digestion of coagulable protein past the coagulable stage (— —O— —), and the liberation of free amino nitrogen (— —+— —). Mixture D, experiment 22.

MIXTURE E.—This showed that the pepsin-hydrochloric acid did not destroy appreciable amounts of the tetanus toxin. The highest final concentration of hydrochloric acid in the toxin-antitoxin mixture injected was 0.005 per cent in experiment 22. In experiments 20 and 21 it was 0.0027 per cent. In these three experiments the dose of mixture E injected into one guinea pig contained but 2 MLD. The deaths of the guinea pigs resulted in the following number of hours after the subcutaneous injection of the mixture: 66, 66, 66, 72, 74, 90.

DESTRUCTION OF ANTHRAX IMMUNE BODIES BY PROTEOLYTIC ENZYMS

Before the writers began the work on tetanus serum, they were engaged in a similar study of anthrax serum. Inoculation experiments were made for the purpose of ascertaining whether the immune bodies in anthrax serum would be destroyed when the serum proteins were digested by pepsin-hydrochloric acid or trypsin-sodium-carbonate. The general method of the inoculation of the guinea pigs, the standardization of the virus, etc. have been described in a previous publication (*6, p. 41*). Chemical analyses were made, similar to those mentioned in the foregoing pages, for the purpose of measuring the extent of pro-

tein splitting and then comparing it with the loss in potency of the serum. The results obtained after 11 inoculation experiments, in which 371 guinea pigs were used, were inconclusive. The digested anthrax serum apparently immunized guinea pigs against anthrax virus as often as it did not. Repeated attempts to obtain definite results were unsuccessful. The main reason for this probably lies in the fact that there is no known quantitative relation between anthrax serum and virus such as there is between tetanus and diphtheria antitoxin and toxin. Unlike the unorganized tetanus and diphtheria toxins, which can not grow or multiply when injected into a test animal, a most carefully standardized dose of anthrax virus, containing a definite number of organisms, can grow to an extent which varies with the resistance of the animal.

SUMMARY

(1) Tetanus antitoxin in 0.5 per cent sodium-carbonate solution was slowly and completely destroyed. At the same time no significant chemical changes in the proteins were detected.

(2) In solutions amphoteric or faintly acid to litmus-paper strips, trypsin destroys the antitoxin, and at the same time the associated proteins are digested. The rates of antitoxin destruction and protein splitting were substantially the same.

(3) The results were the same with solutions containing trypsin and 0.5 per cent sodium carbonate.

(4) Tetanus antitoxin in 0.2 per cent hydrochloric acid was completely destroyed in three or more days. During this time no significant chemical changes in the proteins were detected.

(5) In neutral solutions pepsin did not affect the antitoxin.

(6) In pepsin-hydrochloric acid, proteolysis and antitoxin destruction proceed simultaneously.

(7) These results tend to indicate that tetanus antitoxin is a substance of nonprotein nature. But the stability of the antitoxin is so dependent upon that of the protein to which it is attached, that whenever the protein molecule is split, the antitoxin splits with it.

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RELATION OF THE DENSITY OF CELL SAP TO WINTER HARDINESS IN SMALL GRAINS[1]

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INTRODUCTION

Theoretical considerations suggest a close relation between the density of the cell sap and the ability of plants to survive low temperatures. If death from cold be due to the formation of ice in the plant tissue, to physiological drouth, to the precipitation of the proteids of the protoplasm, or the dessiccation of the protoplasm, then an increase in the electrolytic contents of the sap would increase the hardiness either by lowering the freezing point of the sap or by reducing transpiration.

These considerations led to a study of the sap density of various small grains as one phase of a series of investigations conducted to determine the causes of winter-killing. The work was done during the fall and winter seasons of 1915–16 and 1916–17. Practically all of the actual determinations were made by the junior writer.

EXPERIMENTAL METHODS

The experiments reported in this paper were confined to the winter cereals, rye, wheat, emmer, barley, and oats, which, as shown by general field experience, decrease in comparative winter hardiness in the order named.

Much time was consumed in working out a suitable technic, especially for the extraction of the cell sap. Maceration of the tissue is generally unsatisfactory without a means for centrifuging the sap. As pointed out by Dixon and Atkins,[2] the freezing point of sap extracted without previous treatment of the tissue is likely to be too low. Liquid air was not available for rendering the plasma membrane permeable, as suggested by Dixon and Atkins; hence, we resorted to chemical reagents. For this purpose toluene and chloroform vapor were employed.

This method is not entirely satisfactory because of the slight solubility of these reagents and the opportunity for changes in the cell sap during the rather long period of treatment that is necessary. However, it is believed the methods finally worked out circumvent these objections in a large degree, and that the experimental errors involved are too small to affect the conclusions reached in this investigation.

All determinations were from the leaves of plants sowed the last week in September or the first week in October. The samples were gathered in duplicate, placed immediately in air-tight cans, and taken to the laboratory. All lots for any one experiment were gathered at the

[1] Contribution 15 from the Department of Agronomy, Kansas Agricultural Experiment Station.

[2] Dixon, H. H., and Atkins, W. R. G. osmotic pressures in plants. I. methods of extracting sap from plant organs. *In* Notes Bot. School, Trinity Coll., Dublin, v. 2, no. 4, p. 154–176. 1913.

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same time of day and with the same conditions, as far as possible. In all cases, except when otherwise noted, the leaves were placed on ice or in a refrigerator at a temperature of 4° to 5° C. The extracted sap was kept on ice in tightly stoppered bottles, or in a refrigerator until a determination of the freezing point was made.

The freezing point was determined with a Beckman thermometer and the usual freezing-point apparatus, except that a tube somewhat smaller than usual was employed so that only a small quantity of sap (5–6 c. c.) was necessary for a determination.

The sap was extracted by pressure applied to the leaves which were first macerated, or treated with chloroform or toluene, as indicated.

EFFECT OF TIME OF TREATMENT ON DENSITY OF EXTRACTED SAP

In practically all cases in which different varieties or kinds of grain were compared, determinations were made in duplicate, and in nearly all cases considerable variation between duplicate samples was found. At first this was thought to be due to imperfect extraction of the sap. Since the sap first expressed has a lower density than that extracted later, as shown by Dixon and Atkins and confirmed by us, imperfect extraction might have considerable effect on the density. To avoid this error, experiments were conducted in which the leaves were treated for various periods with chloroform or toluene. Wheat (*Triticum* spp.) leaves were used in the experiments with chloroform, and wheat and barley (*Hordeum* spp.) leaves in those with toluene. In part of the experiments with chloroform duplicate samples were kept during treatment at both room temperature and in an ice box at a temperature of 4° to 5° C. The results are shown in Tables I and II.

TABLE I.—*Effect on sap density of treating wheat with chloroform for different periods and at different temperatures*

Period of exposure.	Freezing point of sap after treatment (°C.).				
	Room temperature.	Room temperature.	Ice box.	Room temperature.	Ice box.
Hours.					
0.25	−0.638	.....	.....	.....	.....
.5	−1.205	.....	.....	.....	.....
1.0	−1.325	−1.325	−0.913	.....	.....
1.5	−1.510	.....	.....	.....	.....
2.5	.....	−1.525	−1.400	.....	.....
4.0	.....	−1.600	−1.568	.....	−1.520
5.5	.....	−1.675	−1.545	.....	−1.770
15.5	.....	.....	.....	−1.89	−1.510
21.5	.....	.....	.....	.....	−2.082
23.0	.....	.....	.....	−2.263	−2.170
39.5	.....	.....	.....	−2.040	−1.475
47.0	.....	.....	.....	−1.895	−1.320
63.5	.....	.....	.....	−1.575	−1.940
71.0	.....	.....	.....	−1.396	−2.030
87.5	.....	.....	.....	−1.340	−2.090
95.0	.....	.....	.....	−1.725	−2.035

TABLE II.—*The effect on sap density of treating leaves of barley and wheat with toluene for different periods*

Exposure to toluene.	Freezing point of sap after treatment (°C.).					
	Barley.			Wheat.		
	Sample 1.	Sample 2.	Average.	Sample 1.	Sample 2.	Average.
Hours.						
14–15	−1.295	−0.975	−1.135	−1.230	−1.040	−1.140
22.5–23	−1.515	−1.565	−1.540	−1.575	−1.670	−1.623
37.5–38	−1.310	−1.355	−1.333	−1.335	−1.275	−1.305
46–46.5	[a]−1.725	[a]−1.710	−1.718	−1.320	−1.395	−1.385
61.5	−1.350	−1.315	−1.333	.....	.....	.....

[a] The freezing point of these samples was not determined until several hours after extraction. Molds had then appeared on the sap, and this is perhaps responsible for the large depression.

The results show a gradual increase in the depression of the freezing point with exposure to chloroform and toluene up to about 23 hours.

This would indicate that the plasma membrane had been rendered thoroughly permeable at that time. There appeared to be no marked difference whether the leaves were kept on ice or at room temperature. Since enzymic action would be least at the low temperature, it was thought best to keep the samples on ice during treatment, and this was done in most cases.

EFFECT OF CHLOROFORM AND TOLUENE ON THE DEPRESSION OF THE FREEZING POINT

Since chloroform and toluene are slightly soluble in water, it seemed desirable to determine their effect on the freezing point of the sap as a possible explanation for the variation in duplicate samples. Accordingly, different amounts were added to distilled water, and the freezing point of the solutions determined. The results are presented in Table III.

TABLE III.—*Effect of chloroform and toluene on the freezing point of distilled water*

Solution.	Freezing point (°C.).	Solution.	Freezing point (°C.).
Distilled water	0.000	Toluene, 1 per cent	−0.020
Chloroform, 1 per cent	− .080	Toluene, 5 per cent	− .038
Chloroform, 5 per cent	− .100	Toluene, 10 per cent	− .035
Chloroform, 10 per cent	− .130		

The solubility of chloroform and toluene appears to be too low to affect the freezing point enough to explain the discrepancies observed in duplicate samples. The slight error due to variation in the amount taken up by the sap could scarcely have been an important factor in the results reported later in this paper. This particularly would be true

of those treated with toluene, its solubility being less than that of chloroform.

RELATIVE FREEZING POINT OF THE SAP OF DIFFERENT KINDS AND VARIETIES OF GRAIN

In this study different kinds of cereals known to vary widely in their resistance to winter killing were selected. The first determination was made on November 27, 1915. The leaves were gathered between 8.15 and 9 a. m. The day was cool and cloudy, with an occasional light shower. The leaves were treated with chloroform for 30 minutes. Probably the depression is somewhat less than it would have been had they been treated for a longer period. The sap was placed on ice as soon as extracted, and left until the freezing point was determined. The results are given in Table IV. The varieties are given in this and the following tables in the order of their hardiness as shown by general field experience.

TABLE IV.—*Relative freezing point of the sap of winter cereals on November 27, 1915*

Kind of grain.	Variety.	Freezing point of sap after treatment (°C.).		Average.
		Sample 1.	Sample 2.	
Rye (*Secale cereale*)		−0.985	−1.095	−1.044
Wheat	Kharkof	−1.230	−1.230	−1.230
Do	Fultz	− .982	−1.170	−1.076
Emmer (*Triticum dicoccum*)	Black Winter	− .990	−1.035	−1.012
Barley	Tennessee Winter	−1.125	−1.110	−1.117
Oats (*Avena sativa*)	Culberson Winter	−1.220	−1.178	−1.199

The data can scarcely be said to show a close relation between the sap density and hardiness. Kharkof wheat, which is very hardy, shows the greatest depression of the freezing point, but the depression for Culberson oats, which is the least hardy of any of the winter cereals, is almost as great. The depression for rye, which will survive greater degrees of cold than any other cereal, is much less than for winter oats.

A second determination was made on December 17 and 18, 1915. The leaves were gathered at 1.30 p. m. on the 17th, placed in air-tight cans, and taken to the laboratory. They were then macerated in a mortar, and the sap was filtered and placed on ice until the next day, when the freezing point of each sample was determined. December 17 was clear and cold. The air temperature when the leaves were gathered was −16.9° C. (1.5° F.) and the soil temperature at a depth of 1 inch was −6.1° C. (21° F.). The leaves of many of the plants were badly wilted, indicating inability to secure enough water from the cold and frozen soil to supply that lost by transpiration. The results are presented in Table V.

TABLE V.—*Relative freezing point of the sap of winter cereals on December 17, 1915*

Kind of grain.	Variety.	Freezing point of sap after treatment (°C.).		
		Sample 1.	Sample 2.	Average.
Rye		−1.330	−1.020	−1.175
Wheat	Kharkof	− .840	−1.030	− .935
Do	Fultz	−1.595	−1.300	−1.442
Barley	Tennessee Winter	−1.330	−1.300	−1.320
Oats	Winter Turf	−1.220	−1.300	−1.260
Do	Culberson	−1.470	−1.420	−1.445

As before, there seems to be little relation between the freezing point of the sap and the known ability of the different cereals to survive severe winters. The greatest depression is recorded for Culberson oats and the least for Kharkof wheat.

This may be explained by assuming that the oats are less able to secure sufficient water to supply that lost by transpiration. Hence, the water content of the tissue would be low and the cyroscopic value of the extracted sap high.

A third determination was made on January 10, 1916. The leaves were gathered at 8.30 a. m. The day was cool and cloudy. The soil was wet, but not frozen. The plants appeared to be turgid. The leaves were treated for 22.5 hours with toluene on ice (temperature 3° C.), allowed to stand exposed to the air for ten minutes to permit the excess of toluene vapor to leave the tissue, and then expressed. The sap was kept on ice at a temperature of about 1.0° C. until January 11, when the freezing point was determined. The results are given in Table VI.

TABLE VI.—*Relative freezing point of the sap of winter cereals on January 10, 1916*

Kind of grain.	Variety.	Freezing point of sap after treatment (°C.).		
		Sample 1.	Sample 2.	Average.
Rye		−1.450	.....	−1.450
Wheat	Turkey	−1.360	−1.510	−1.435
Do	Fultz	−1.390	−1.440	−1.415
Barley	Tennessee Winter	− .970	−1.390	−1.180
Oats	Winter Turf	−1.385	−1.525	−1.455
Do	Culberson	−1.540	−1.380	−1.460

In this case also there appears to be little if any relation between the freezing point of the sap and winter hardiness. In fact, with the exception of the barley, the freezing point of all is about the same. There is only 0.01 degree difference in the freezing point of the sap of rye, which is the hardiest of the cereal grains, and of Culberson oats, which is the least hardy.

A test comparing rye, wheat, and barley was conducted on March 8. The leaves were collected at 4.30 p. m. They were treated with toluene for about 16 hours, and the sap was kept on ice until the freezing point was determined. The results are given in Table VII.

TABLE VII.—*Relative freezing point of the sap of winter cereals on March 8, 1916*

Kind of grain.	Variety.	Freezing point of sap after treatment (°C.).		
		Sample 1.	Sample 2.	Average.
Rye		−1.250	−1.615	−1.433
Wheat	Kharkof	−1.945	−2.015	−1.980
Barley	Tennessee Winter	−1.710	−1.765	−1.738

Here also no significant differences were observed. The barley showed a greater depression than the rye, although much less able to survive low temperatures.

A fifth test compared wheat, barley, and oats grown in the greenhouse. The leaves were from plants about 40 days old from planting. They were watered several times before gathering, in order to insure a normal condition with respect to turgidity. The leaves were treated with toluene on ice for 16.5 hours, and the extracted sap was placed on ice until the freezing point was determined. The results, which are presented in Table VIII, show no large differences and apparently no relation between depression of the freezing point and the ability of the different kinds of grain to survive low temperatures.

TABLE VIII.—*Relative freezing point of the sap of winter cereals grown in the greenhouse*

Kind of grain.	Variety.	Freezing point of sap after treatment (°C.).		
		Sample 1.	Sample 2.	Average.
Wheat	Kanred	−1.700	.....	−1.700
Barley	Tennessee Winter	−1.840	−1.780	−1.810
Oats	Culberson	−1.595	−1.755	−1.675

Several determinations were made directly without extracting the sap. This was done by wrapping the thermometer bulb with leaves, placing it in the air chamber, and inserting the whole in the freezing mixture of salt and ice, and proceeding in the usual way. This method appears to have been used by Müller-Thurgau and more recently by Bouyoucos and McCool.[1]

[1] BOUYOUCOS, G. J., and MCCOOL, M. M. DETERMINATION OF CELL SAP CONCENTRATION BY THE FREEZING POINT METHOD. *In* Jour. Amer.-Soc. Agron., v. 8, no. 1, p. 50. 1916.

Owing to the time required to make readings, only two or three varieties could be examined at one time. The data are presented in Table IX. Each recorded reading is the average of several from the same sample.

TABLE IX.—*Relative freezing point of the leaves of winter cereals*

Date of determination.	Freezing point (°C.).			
	Kharkof wheat.	Fultz wheat.	Rye.	Barley.
January 16, 1917	.....	.....	−3.56	−2.59
January 19, 1917	−3.47	.....	−3.58	.....
January 27, 1917	−2.17	−2.06	−2.10	.....

The depression of the freezing point determined in this way is somewhat greater than for the extracted sap, but the differences between kinds of grain are not consistent enough to certainly indicate a relation between the freezing point of the sap and ability to resist cold. In two tests the rye has a lower freezing point than either barley or wheat; but in a third test, on January 27, Kharkof winter wheat showed a greater depression than the rye.

An attempt was made to obtain a measure of the accuracy of this method by collecting four samples of winter wheat at the same time from the same plot, and treating them identically. The freezing point of each is shown in Table X.

TABLE X.—*Freezing point of duplicate samples of wheat determined by the direct method*

Sample No.	Freezing point (°C.).
1	−3.66
2	−3.85
3	−3.57
4	−3.53
Average	−3.65

It will be seen that the variations are of the same order as those observed in determinations of the extracted sap.

Table XI summarizes the data from all tests. Since the depression of the freezing points appears to depend so much on the balance the plants are able to maintain between absorption and transpiration, it would manifestly be unfair to include any tests but those in which the leaves were secured under like conditions. Hence, the average includes the first three tests only.

TABLE XI.—*Summary of the relative freezing points of the leaves and leaf sap of various winter cereals*

Date.	Freezing point (° C.)						
	Rye.	Kharkof wheat.	Fultz wheat.	Black Winter emmer.	Tennessee Winter barley.	Winter Turf oats.	Culberson Winter oats.
Nov. 27, 1915	−1.044	−1.230	−1.076	−1.012	−1.117	.....	−1.199
Dec. 17, 1915	−1.175	−.935	−1.442	.....	−1.320	−1.260	−1.445
Jan. 10, 1916	−1.450	−1.435	−1.415	.....	−1.180	−1.455	−1.400
Mar. 8, 1916	−1.433	−1.980	.....	.....	−1.738	.....	.....
Jan. 16, 1917	−3.560	.....	.....	.....	−2.590	.....	.....
Jan. 19, 1917	−3.580	−3.470	.....	.....	.....	.....	.....
Jan. 27, 1917	−2.100	−2.170	−2.000	.....	.....	.....	.....
Average [a]	−1.223	−1.200	−1.311	.....	−1.208	.....	−1.334

[a] Average of determinations of November 27, December 17, and January 10, only.

The average for the three tests show practically the same depression of the freezing point for rye, Kharkof wheat, and Tennessee Winter barley, it being decidedly less for these grains than for Fultz Winter wheat and Culberson Winter oats. As mentioned in connection with the data for each test, the depression of the freezing point seems to depend more on the conditions of the leaves with respect to turgidity than on variety characteristics.

RELATION OF TURGIDITY TO DENSITY OF SAP AND RESISTANCE TO LOW TEMPERATURE

Because of the apparent relation between turgidity and sap density observed in these experiments, it seemed desirable to investigate this phase more thoroughly. Accordingly seedlings of hard wheat (Turkey), soft wheat (Fultz), and winter oats (Culberson), grown in the greenhouse were removed from the soil and one set was exposed to sunlight at room temperature from two to three hours and another set placed in the same location as the first but with the roots immersed in water. The freezing point of the leaves was then determined by the direct method. The results are shown in Table XII.

TABLE XII.—*Relation of turgor to the freezing point of leaves*

Variety.	Freezing point (°C.).					
	Turgid.			Wilted.		
	No. 1.	No. 2.	Average.	No. 1.	No. 2.	Average.
Hard wheat (Turkey)	−1.280	−1.630	−1.405	−1.91	−1.08	−1.045
Soft wheat (Fultz)	−1.510	−1.105	−1.308	−1.93	.....	−1.930
Winter oats	−1.180	−1.280	−1.230	−2.34	−1.98	−2.160
Average	.....	.....	−1.314	.....	.....	−2.012

The results show beyond doubt the effect of turgidity on the freezing point of the extracted sap, the difference between wilted and turgid leaves being decidedly greater than was observed between different kinds of grain.

Further evidence is furnished by experiments conducted during the winter of 1915–16 in which wilted and turgid plants were exposed for different periods to freezing temperatures. Lots of 15 plants each were treated in four different ways before exposure to cold as follows: (1) Exposed to air for 30 minutes, (2) kept in air-tight cans for 30 minutes, (3) roots in water for 10 minutes, and (4) entire plant submerged in water for 10 minutes. All plants were then placed in a chamber surrounded with salt and ice in which the temperature was from −2.0° to −3.0° C. Portions of each lot were removed at intervals of 10, 20, and 30 minutes and placed with their roots in water to test their ability to recover. The results are shown in Table XIII.

TABLE XIII.—*Relation of turgidity to cold resistance*

Period of freezing.	Effect on plants.			
	Lot 1, exposed to air.	Lot 2, kept in air-tight cans.	Lot 3, roots in water.	Lot 4, plants submerged in water.
Minutes.				
10	Not injured	Not injured	Slightly injured	Slightly injured.
20	do	Slightly injured	Killed	Killed.
30	Slightly injured	Killed	do	Do.

In all cases the plants which were submerged in water or whose roots were in water were killed or injured decidedly more than those which were exposed to the air previous to freezing.

In another experiment conducted in a similar manner the plants were exposed to the air at greenhouse temperature for different periods of time and then exposed to a temperature of −6.5° C. for about 10 minutes. One set was exposed to the air for 2.5 hours, another for 1.5 hours, another for 1 hour, and another for 30 minutes before freezing. Plants which had not been exposed to the air and others that had been exposed to the air for 2.5 hours but which had not been frozen were included as controls. All which had been wilted for 1.5 hours or longer survived with no apparent injury, while all turgid plants and those wilted for 1 hour or less were entirely killed. The appearance of typical plants from each lot after treatment is shown in Plate 53.

SUMMARY

To summarize briefly, there appears to be no relation between the cryoscopic value of the extracted sap of winter rye, wheat, emmer, barley, and oats grown in the field with normal conditions and their

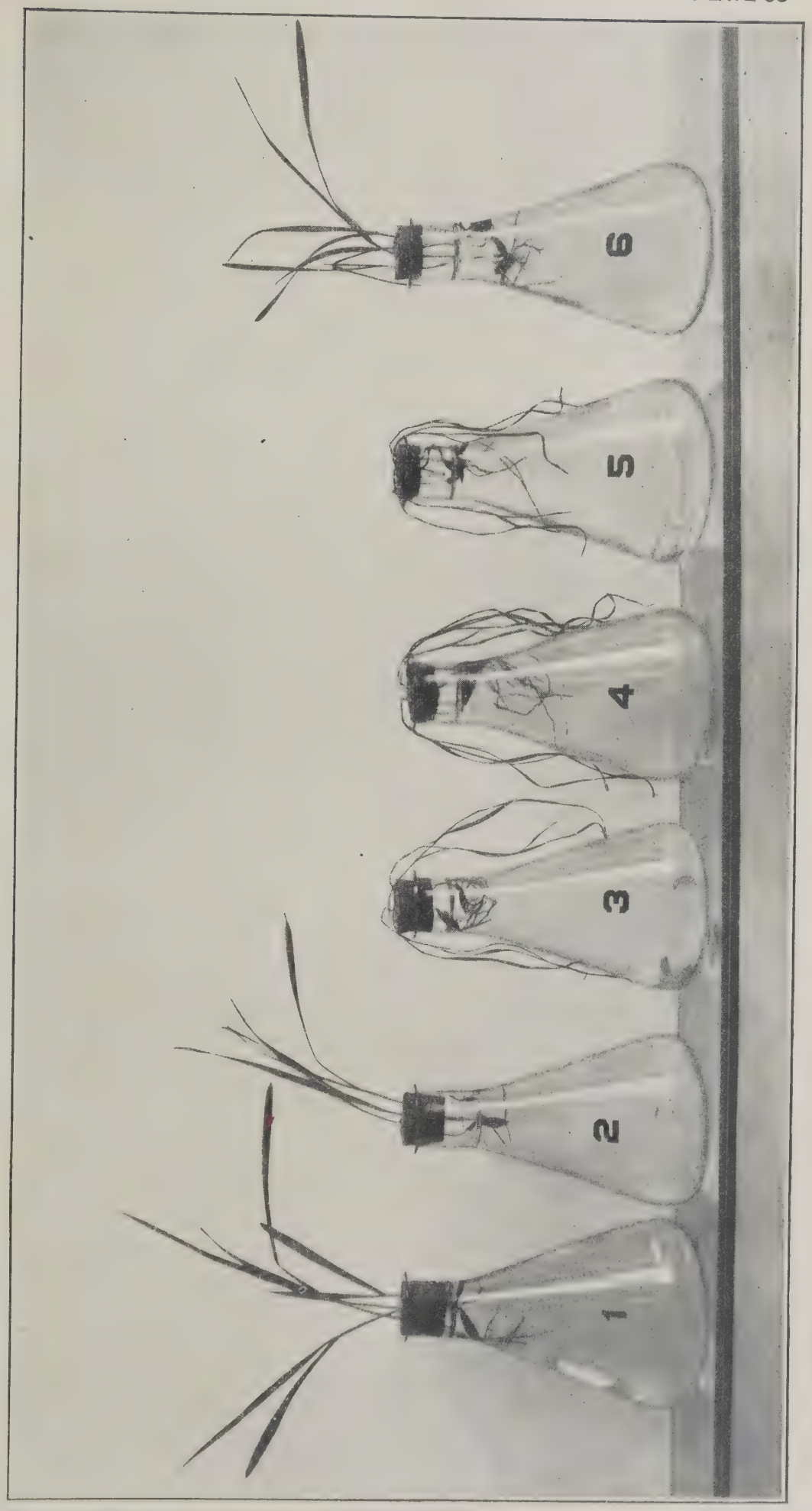
ability to resist winterkilling. Turgidity of the tissue as influenced by physiological drouth appears to have more influence than the kind of grain on the concentration of the cell sap.

On the other hand, for tender plants of the same varieties grown in the greenhouse there appears to be a definite relation between the freezing point of the cell sap, the turgidity of the tissue, and resistance to low temperature. This relation for plants grown in the field perhaps also holds true in the fall and early winter and following periods of mild weather during the winter.

PLATE 53

Effect of wilting on ability of small grains to survive low temperatures:

Flask 1.—Exposed to air for 2.5 hours previous to freezing.
Flask 2.—Exposed to air for 1.5 hours previous to freezing.
Flask 3.—Exposed to air for 1 hour previous to freezing.
Flask 4.—Exposed to air for 0.5 hour previous to freezing.
Flask 5.—Not exposed to the air previous to freezing.
Flask 6.—Exposed to the air for 2.5 hours, but not frozen.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INFLUENCE OF TEMPERATURE AND PRECIPITATION ON THE BLACKLEG OF POTATO

By J. ROSENBAUM, *Assistant Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture,* and G. B. RAMSEY, *Assistant Pathologist, Maine Agricultural Experiment Station*

OVERWINTERING IN THE SOIL

It is still a question in the minds of some pathologists whether the blackleg organism,[1] *Bacillus phytophthorus* appel, can remain in the soil over a winter and start the disease the following spring. In order to test this point, about a bushel of potato tubers (*Solanum tuberosum*), badly diseased with the blackleg, were collected and placed at the usual depth, about 2 or 3 inches apart, on October 11, 1915, in Aroostook County, Maine. In the spring of 1916, at the time when the general crop of potatoes was being planted, some Irish Cobbler and Spaulding Rose tubers were carefully selected, disinfected in the usual manner, and planted in the rows where the diseased potatoes had been planted the previous fall. When these were being planted a large number of overwintered tubers were found in the soil, either wholly or partially decayed. A careful watch was kept of the plants throughout the growing season. In neither the Irish Cobbler, an early-maturing variety, nor the Spaulding Rose, a late-maturing variety, was a single case of blackleg developed.

In the fall of 1916 a similar experiment was begun. The same soil was used, but instead of naturally infected tubers being used, approximately 3 pecks of Irish Cobbler potatoes were inoculated by means of a hypodermic syringe with a very virulent culture of the blackleg organism. The inoculated tubers were then placed in a covered container and allowed to remain there until each of them showed a marked rot at the point of inoculation. The tubers were then planted whole, about 1 foot apart in a trench 5 inches deep. On May 18, 1917, this row was opened with a hand hoe, care being taken not to disturb the decayed tubers any more than necessary. Healthy Irish Cobbler potatoes, known to be free from blackleg, were cut in the usual way, and one seed piece was placed in the old seed bed with each of the decayed, overwintered tubers. A second row was made by the side of the one mentioned and planted with the same

[1] The blackleg organism has been generally referred to as *B. phytophthorus* Appel. According to the works of Morse (MORSE, W. J. STUDIES UPON THE BLACKLEG DISEASE OF THE POTATO, WITH SPECIAL REFERENCE TO THE RELATIONSHIP OF THE CAUSAL ORGANISMS. *In* Jour. Agr. Research, v. 8, no. 3, p. 79–126. 1917.) all cultures of blackleg isolated by him in Maine agree with *B. solanisaprus* Harrison, but for certain reason, he believes the name *B. atrosepticus* Van Hall should be adopted. According to the work of Smith (SMITH, Erwin F. BACILLUS PHYTOPHTHORUS APPEL. *In* Science, v. 31, no. 802, p. 748–749. 1910.), *B. phytophthorus* and *B. solanisaprus* are different.

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kind of healthy seed to serve as a control. Each of these rows was 110 feet long. No blackleg showed in the plants during the growing season, and at digging time, September 10, the tubers were healthy and normal in every respect.

A similar experiment was carried out at Norfolk, Va.[1] Half a barrel of artificially infected tubers were thoroughly rotted by the blackleg organism before they were placed in the soil. There is no question that the bacteria were introduced into the soil in large numbers. The following spring when the potatoes were planted, some of the rotted tubers were still to be found in the soil. No blackleg appeared throughout the course of the experiment.

As supplementary tests to ascertain whether the organism still remained alive in the soil where the diseased tubers were planted, in each of the three experiments mentioned above isolations were made from the tubers themselves and in two of the experiments from the soil immediately surrounding the tubers. Many series of these isolations were made from various parts of the plots, and a great number of bacteria were obtained. All of these were tested for their ability to produce blackleg by innoculating healthy, growing plants and cut slices of healthy tubers. As a control for these inoculations, two different strains of authentic cultures of blackleg were used. The results of this work showed that the bacteria isolated from the soil were unable to produce the disease, while the two control cultures did produce the typical blackleg symptons.

In no case, therefore, was the blackleg organism found to live over a winter in the soil or in the tubers remaining in the soil. Morse [2] says:

> Observations in Maine indicate that under the climatic conditions which exist there infected seed potatoes are the sole source of infection and distribution and that the disease does not live over the winter in the soil.

In order to get an idea as to whether the results described above are normal, or whether the winter of 1916 was unusually severe, data in regard to the temperature, amount of snowfall, and total precipitation, were collected for three consecutive winters, as shown in Table I. Examination of this table shows that the winter of 1915–16 was not an unusual one. The average mean minimum and maximum during that season was slightly less than for the winter of 1914–15 but more than for that of 1913–14. The snowfall in inches in 1915–16 was considerably more than in 1914–15 and only slightly less than during 1913–14. This data would seem to indicate that the blackleg organism under the winter conditions that exist in northern Maine cannot survive in the soil or in the diseased tubers remaining in the soil.

[1] The work at Norfolk was done in cooperation with the Virginia Truck Experiment Station and was in charge of Mr. J. A. McClintock.

[2] Morse, W. J. Op. cit., p. 91.

TABLE I.—*Temperature and precipitation at Presque Isle, Me., 1913–1916*

Month.	1913–14				1914–15				1915–16			
	Temperature.		Precipitation.		Temperature.		Precipitation.		Temperature.		Precipitation.	
	Mean.				Mean.				Mean.			
	Minimum.	Maximum.	Snow.	Total.	Minimum.	Maximum.	Snow.	Total.	Minimum.	Maximum.	Snow.	Total.
	°F.	°F.	Inches.	Inches.	°F.	°F.	Inches.	Inches.	°F.	°F.	Inches.	Inches.
November	22	44	2.65	0.71	18.5	36.8	12	3.5	24.2	38.3	9	2.19
December	13	27	27	3.49	4.4	24.1	18	2.08	14.3	28.5	33.5	4.05
January	−9.4	17.1	29	2.95	2.0	23.5	17.5	2.75	−.67	21.5	21	2.2
February	−13.1	16.4	23	2.7	6.3	28.8	22	4.1	−1.1	20.6	18.5	2.35
March	16.5	34.7	15	2.5	13.8	33.2	13	1.4	4.4	28.7	11	1.1
April	19.4	44.3	25	4.0	29.3	49.7	Trace	3.4	29.1	53.4	17	1.75
Average	8.06	30.58	20.27	2.72	12.38	32.68	13.74	2.87	11.70	31.8	18.3	2.27

The experiment at Norfolk also seems to indicate that the organism does not overwinter in the soil, even under climatic conditions much milder than those found in Maine.

BLACKLEG AS FOUND IN NORTHERN MAINE IN 1914, 1915, AND 1916

During the summer of 1914, following a winter with an average mean minimum temperature of 8.06° F., an average mean maximum of 30.58°, and an average monthly precipitation of 2.72 inches, the blackleg was very prevalent in the potato fields under observation in Aroostook County. It was then attributed to a variety of conditions. However, no actual counts showing the percentage of the disease were made. The following year (1915) blackleg was likewise very prevalent in the same and other fields. This was also true in commercial fields where disinfection but not careful selection of the seed was practiced. The Irish Cobbler and Spaulding Rose were attacked as severely as any of the other varieties. For this reason the observations recorded here for 1915 and 1916 have been made on these two varieties. During these two summers a number of fields were inspected, and counts made as to the percentage of infected plants. As far as possible, counts were made on the same fields in 1915 and 1916. In all cases the seed used in 1916 was the progeny of the fields from which the counts were made in 1915. The results are presented in Table II. The significant thing about the results is the great difference in the percentage of blackleg found during 1915 and 1916. There is a striking uniformity in regard to this difference in six different fields from two varieties, an early and a late one. The counts were made approximately on the same date in both summers, and as far as possible, the same treatment and cultural conditions were employed both seasons. By examining the percentages of disease in the totals as given in Table II for the Spaulding Rose, it will be seen that in 1915 the percentage of blackleg is 8.47, 7.52, and

7.77, as compared to 0.84, 1.96, and 1.00 per cent in 1916 for the three fields, an average difference of 6.65 per cent. The data for the Irish Cobbler show 4.04, 1.57, and 5.79 per cent in 1915, as compared to 2.1, 1.57, and 1.17 per cent in 1916 for the three fields, an average difference of 2.19 per cent.

TABLE II.—*Prevalence of blackleg of Irish potatoes at Presque Isle, Me., during 1915 and 1916*

SPAULDING ROSE

Date of count.	Field No.	1915			1916		
		Number of healthy hills.	Number of diseased hills.	Percentage of disease.	Number of healthy hills.	Number of diseased hills.	Percentage of disease.
June 30	1	224	6	2.61	1,500	12	0.79
July 14	1	165	22	11.76	1,490	15	1.00
August 23	1	162	23	12.43	300	1	.33
Total	.....	551	51	8.47	3,290	28	.84
June 30	2	200	9	4.31	312	12	3.70
July 14	2	196	18	8.41	290	3	1.02
August 23	2	256	26	9.22	400	5	1.23
Total	.....	652	53	7.52	1,002	20	1.96
June 30	3	200	15	6.98	600	12	1.96
July 14	3	297	27	8.33	480	2	.41
August 23	3	310	26	7.74	300	0	0
Total	.....	807	68	7.77	1,380	14	1.00

IRISH COBBLER

		1915			1916		
June 30	1	290	6	2.03	916	12	1.29
July 14	1	312	14	4.29	400	18	4.31
August 23	1	300	18	5.66	317	5	1.55
Total	.....	902	38	4.04	1,633	35	2.10
June 30	2	400	2	.50	400	4	.99
July 14	2	360	7	1.91	388	12	3.00
August 23	2	366	9	2.40	400	3	.74
Total	.....	1,126	18	1.57	1,188	19	1.57
June 30	3	200	5	2.44	200	10	4.76
July 14	3	200	22	9.91	517	2	.39
August 23	3	300	16	5.06	300	0	0
Total	.....	700	43	5.79	1,017	12	1.17
Total for 3 fields	.....	2,728	99	3.5	3,838	66	1.69

In general, the results presented in Table II for the years 1915 and 1916 are wholly comparable and give one an idea as to the difference in the relative prevalence of blackleg throughout Aroostook County dur-

ing the two seasons. Aside from the counts as given, careful watch was kept of a large number of fields throughout the county and in all cases there was a surprisingly small amount of blackleg in 1916 as compared to 1915. This was especially true during the month of August. This also explains why there is a greater difference shown in the Spaulding Rose than in the Irish Cobbler. The former, being a late variety, generally shows a greater number of plants diseased with the blackleg in August than the latter. In 1915 the Spaulding Rose showed a number of affected plants during August, but in 1916 few or none were seen during the same month. With the Irish Cobbler, on the other hand, blackleg makes its appearance in the latter part of June and early July and usually continues in evidence up into the first part of August. By this time potatoes begin to approach maturity, and very few additional plants show symptoms of the blackleg.

The writers wish to call attention to one exception found in 1916. On a one-six-acre plot of Irish Cobbler tubers used in connection with another experiment, a portion showed approximately 8 per cent of blackleg during August. This part of the plot was very low and wet, compared with the adjoining plots, and, as will be seen later, this explains the large percentage of diseased plants.

TEMPERATURE AND PRECIPITATION DURING 1914, 1915, AND 1916.

Morse[1] makes the following statement regarding the influence of weather conditions on blackleg:

> The progress of the disease is markedly influenced by weather conditions. . . . . More blackleg is observed in wet than in dry seasons.

and further—

> Soil conditions also are factors which influence outbreaks of blackleg. All other things being equal, the disease is more likely to occur in wet than in dry soil, and is more prevalent when the early part of the growing season is characterized by abundant rainfall.

The great difference in the severity of the disease found in 1914, 1915, and 1916 can be explained by differences in temperature and precipitation found during the three growing months, June, July, and August, during these three years, especially as it existed in August. In Table III are given the mean maximum and the mean minimum temperature, and the precipitation. Averaging the three months, it is seen that both the mean maximum and the mean miminum temperatures in 1916 were higher than in 1915 or 1914, while the precipitation for the same year was less than in the two preceding years. During the month of August, which showed the greatest differences in the percentages of the disease, the mean maximum in 1916 was 79° F., while in 1915 it was 72.7°, a difference of 6.3°; the mean minimum in 1916 was 64.8° as compared to 50.9° in 1915, a difference of 13.9°. The higher maximum as well as the

[1] Morse, W. J. op. cit., p. 84-85.

higher minimum in 1916 as compared with 1915 during the month of August makes the actual difference in temperature much greater than is indicated by the above figures.

TABLE III.—*Temperature and precipitation records at Presque Isle, Me.*

Month.	1914.			1915.			1916.		
	Mean temperature.		Precipitation.	Mean temperature.		Precipitation.	Mean temperature.		Precipitation.
	Maximum.	Minimum.		Maximum.	Minimum.		Maximum.	Minimum.	
	°F.	°F.	Inches.	°F.	°F.	Inches.	°F.	°F.	Inches.
June	69.1	42.9	4.8	73.7	47.6	1.95	70.1	49.2	2.16
July	77.6	48.5	2.23	78.0	50.2	3.4	80.0	50.0	3.6
August	74.1	46.2	2.35	72.7	50.9	3.5	79.0	64.8	1.7
Average	73.6	45.8	3.12	74.8	49.5	2.95	76.3	54.6	2.48

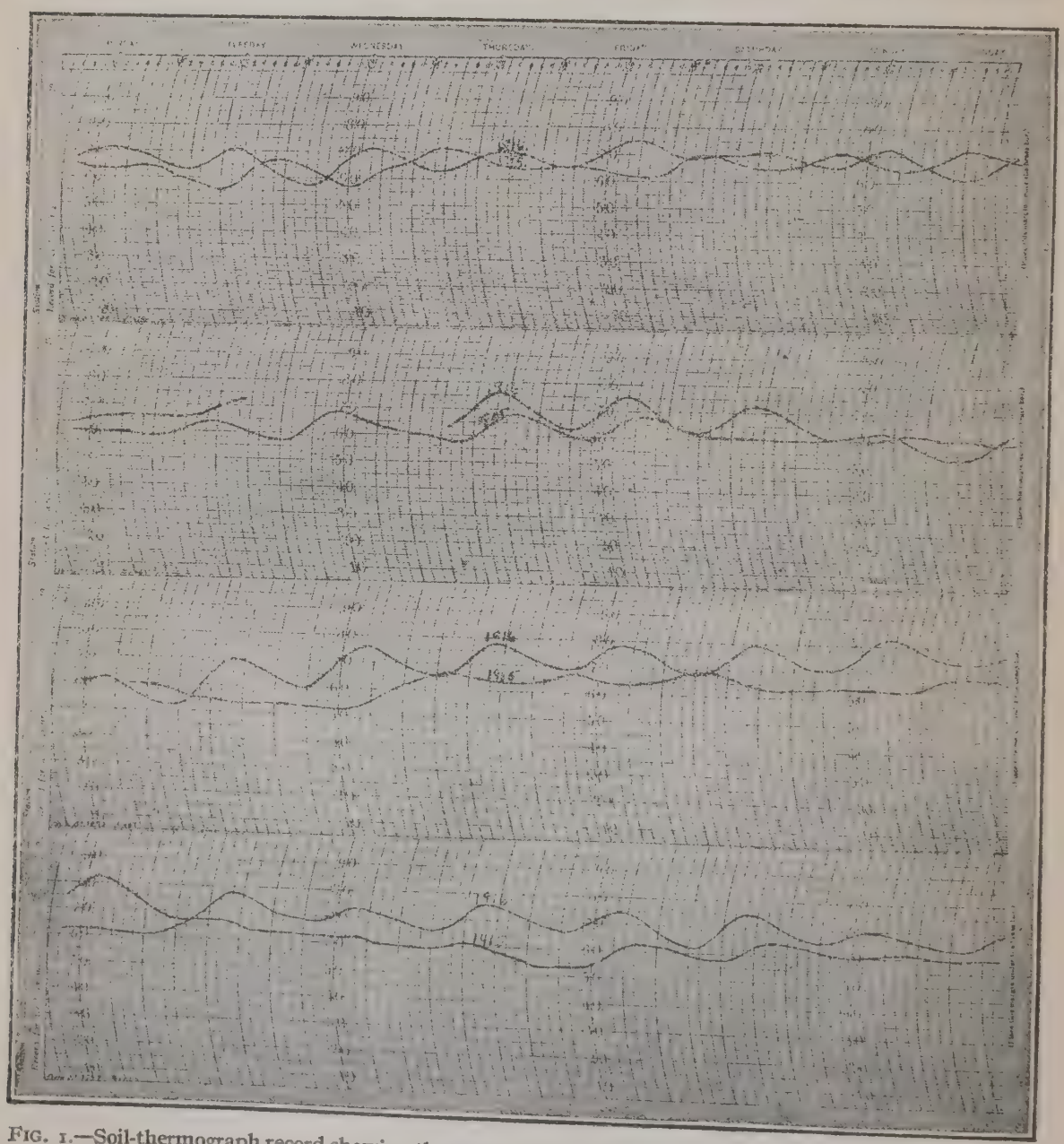

FIG. 1.—Soil-thermograph record showing the range in temperature for the month of August, 1915 and 1916 at Presque Isle, Me.

Heretofore it has been shown that there was a great difference in the air temperature during the two summers of 1915 and 1916. In figure 1 is shown a thermograph record of the soil temperature as recorded for the month of August in 1915 and 1916. These records were obtained by burying the thermograph bulb at the depth at which potatoes are planted, about 5 inches, at practically the same spot in both 1915 and 1916. An examination of this figure shows that in 1915, during the month of August, the soil temperature fluctuated between 60° and 70° and dropped below 60° twice during the latter half of the month. In 1916 the temperature was more fluctuating than for the same month the previous year and varied from 60° to 70° the early part of the month, reaching a maximum of 70° to 80° the last part of the month.

A close correlation therefore appears to exist between high temperature and little rainfall with a small percentage of blackleg, and vice versa.

SUMMARY

(1) No evidence could be obtained to indicate that the blackleg organism, under the winter conditions that existed during 1915–16 and 1916–17 in Aroostook County, Me., and during 1916–17 at Norfolk, Va., can live over in the soil or in diseased tubers that may remain there.

(2) Weather records show that the winter of 1915–16 was not an unusual one for Aroostook County.

(3) The severity of the disease during the growing season is closely correlated with temperature and precipitation and is dependent upon them. A high temperature and low precipitation tend to diminish the disease, while a low temperature and high precipitation produce conditions favorable for it.

A NEW BACTERIAL DISEASE OF GIPSY-MOTH CATERPILLARS[1]

By R. W. Glaser,

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INTRODUCTION

During the summer of 1915 a large series of eggs of the Japanese gipsy moth (*Porthetria dispar* Linnaeus) were hatched. These eggs had been obtained for the writer by Prof. Richard Goldschmidt from Ogi, Japan. On reaching the third stage many of the caterpillars began to die of a peculiar disease which the writer had never in previous years noticed in any of his American cultures. The infection later spread to the American race, and the most rigorous methods of isolation and disinfection had to be inaugurated in order to save most of the cultures from extinction. The disease was very soon controlled, and this led at once to a belief in its bacterial origin, and its distinctness from wilt (polyhedral disease).[2] Anyone who has worked with wilt knows with what great difficulty this disease is controlled once an epidemic gains a foothold.

Inspired by the belief that this new disease might be used in combating the gipsy moth in the field, the writer made a systematic study of it during the seasons of 1915, 1916, and 1917.

SYMPTOMS AND CHARACTERISTICS OF THE DISEASE

When a caterpillar contracts this new disease, which may be provisionally named the "Japanese gipsy-moth disease," it acquires a violent form of diarrhea, loses its appetite, and finally ceases to eat. The insect seems to lose all muscular coordination and usually crawls slowly to some elevated place, where it soon dies. After death it hangs in a flaccid manner by its prolegs, with an appearance of death from wilt. In contradistinction to wilt, however, the skin does not rupture, but is so tough that one can pick up and stretch the animal with considerable force before the skin breaks.

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[1] Contribution from the Bureau of Entomology, U. S. Department of Agriculture, in cooperation with the Bussey Institution of Harvard University (Bussey Institution No. 140).

[2] Glaser, R. W. WILT OF GIPSY-MOTH CATERPILLARS. *In* Jour. Agr. Research, v. 4, no. 2, p. 101–128, pl. 11–14. May 15, 1915. Literature cited, p. 127–128.

Chapman, J. W., and Glaser, R. W. FURTHER STUDIES ON WILT OF GIPSY MOTH CATERPILLARS. *In* Jour. Econ. Ent., v. 9, no. 1, p. 149–163. 1916.

Glaser, R. W., and Chapman, J. W. THE NATURE OF THE POLYHEDRAL BODIES FOUND IN INSECTS, *In* Biol. Bul., v. 30, no. 5, p. 367–390. May, 1916. Bibliography, p. 383–384.

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A microscopic study of smears from the dead animals readily precludes the possibility of wilt. At times an animal will contract wilt as well as the Japanese disease, but such cases are exceptional. Therefore, when caterpillars which die from the new disease are examined, polyhedra are not found, but large numbers of a streptococcus which the writer has described under the name "*Streptococcus disparis*" are present. Sections demonstrate that this bacterium, during the early stages of the disease, is found throughout the alimentary tract. Later, and especially after death, the intestinal epithelium disintegrates and ruptures, liberating the organisms into the body cavity, where they invade practically all the tissues. Naturally species of bacteria different from *S. disparis* are also quite frequently found in the gut (intestinal flora), so that a pure culture cannot be obtained by inoculating culture tubes with material from the stomach or intestines.

During the earlier stages of the disease, when the animals contract diarrhea, the semiliquid feces everywhere soil the food plants. This fecal matter is full of the microorganism in question, and is the principal cause for the rapid spread of the infection.

The writer has isolated *S. disparis* many times from cases of the Japanese disease and has never failed to reproduce the malady. The organism was always again recovered by plating and other successful infections performed with the pure culture.

LABORATORY EXPERIMENTS

Inoculation experiments were not thought necessary, for the reason that the bacterium does not originally invade the blood of the insect. *S. disparis* invades the alimentary tract with the ingested food, and therefore food-infection experiments were considered more instructive.

Tables I to III are self-explanatory. The animals used in the experiments came from a selected stock of American caterpillars from which the wilt disease had been nearly eliminated. Gipsy-moth caterpillars taken directly from the field can not be used for experimentation, for large numbers are invariably infected with wilt. A stock of caterpillars comparatively free from this disease must first be produced by selection and this, as the writer has shown in a previous publication, takes at least three years. Each animal used in the streptococcus experiments was isolated in a separate sterile glass fruit jar and the food foliage was shipped daily from a region where the gipsy moth does not occur. This latter precaution was taken in order to preclude the introduction of wilt. The nutrient bouillon cultures were diluted one-half with sterile water and sprayed on the foliage by means of a fine atomizer until the leaves were visibly wet. Controls accompanied all of the experiments and are absolutely necessary to the proper interpretation of all disease experiments with insects.

TABLE I.—*Results of infection of eight fourth-stage American Porthetria dispar larvæ fed with red-oak leaves sprayed with a 72-hour bouillon culture of Streptococcus disparis. Bacterium isolated from alimentary tract of animal which had died from disease* [a]

Number of days	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of deaths	....	....	....	....	....	....	....	2	2	....	....	....	1

[a] Three pupated and emerged. Eight controls accompanied this series. All lived, pupated, and emerged. Five dead animals tested and *S. disparis* recovered.

TABLE II.—*Results of infection of eight fourth-stage American Porthetria dispar larvæ fed with red-oak leaves sprayed with a 72-hour bouillon culture of Streptococcus disparis recovered from one of the dead animals used in previous experiments* [a]

Number of days	1	2	3	4	5	6
Number of deaths	.....	1	1	1	2	1

[a] Two pupated and emerged. Eight controls accompanied this series. All lived, pupated, and emerged. Six dead animals tested and *S. disparis* recovered.

TABLE III.—*Results of infection of 25 third- and fourth-stage American Porthetria dispar larvæ fed with apple leaves sprayed with a 72-hour bouillon culture of Streptococcus disparis.* [a]

Number of days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Number of deaths	...	...	...	...	...	2	...	2	1	1	9	4	3	2	....	1

[a] Twenty-five controls accompanied this series. One died of wilt; the remaining ones lived, pupated, and emerged. In the infection experiment the two which died on the sixth day succumbed to wilt (polyhedral disease); the remaining ones died of the new disease. The one which died on the ninth day, the three on the eleventh day, the two on the thirteenth day, and the one on the sixteenth day were tested and *S. disparis* recovered. The remaining dead were merely examined microscopically.

At the conclusion of each experiment it is stated that the dead animals were "tested." This signifies that stained smears were studied, the material was "plated out" on agar, and the species of bacteria isolated was observed culturally and biochemically. (See description of *S. disparis* on pages 520–521.)

From an examination of Tables I to III it will be seen that the period from infection to death varies considerably. This is probably due to individual resistance or to differences in the number of organisms ingested. Death may be expected any time after about 24 hours; indeed, it may be postponed for as long as 16 days. In Experiment I three and in Experiment II two animals lived and transformed into moths. At present no definite explanation can be offered for this occurrence. These five caterpillars became infected because the contaminated food was never removed until nearly all of it had been eaten. By confining the caterpillars in glass fruit jars having tin screw tops the food foliage can be kept palatable to the animals for three and sometimes four days.

The writer performed three times as many infection experiments as those outlined, but since the results were similar, a repetition here would be superfluous.

In a large experiment like the one given in Table III it is hopeless to analyze thoroughly every death, on account of the enormous work involved; but it is hoped that the number carefully studied will represent a fair sample of all the deaths. Of course every dead caterpillar was examined microscopically, but by careful analysis is meant the systematic study of all species of bacteria isolated.

An attempt was made to infect 10 silkworms (*Bombyx mori* Linnaeus) and 10 army worms (*Cirphis unipuncta* Haworth) with *S. disparis*, but the organism does not seem to be pathogenic to these two insects. Guinea pigs and rabbits were forced to drink pure cultures of the organism, but failed to develop any distressing symptoms. If the disease is to be introduced into the field, it appears highly important to determine whether the organism would prove pathogenic to mammals like horses, cows, pigs, dogs, and even to human beings. After the guinea pig and rabbit experiment the writer mustered enough courage to drink 5 c. c. of a pure bouillon culture of *S. disparis*. Up to the date of writing, about seven months, he has failed to notice any distressing symptoms.

PATHOLOGY

The pathology of the Japanese gipsy-moth disease is quite interesting and distinct. Of course during the later stages of the disease practically all of the tissues are affected, but the most striking early changes occur in the muscle tissues. Plate 54, A, is a reproduction of a photomicrograph of normal and early pathological muscle tissue. The strands to the right and left are normal, whereas the muscle strand in the middle does not show the striæ so clearly, and the individual fibrillæ seem to be more loosely arranged. Plate 54, B, shows a later stage in the progress of the disease. Here the muscle tissue has lost its striated appearance, which, as can be seen, is due to the fact that the fibrillæ have lost their compactness and have separated from one another like threads of cotton. The sarcolemma disintegrates gradually with the rest, and the nuclei of the cells lose their normal positions and become scattered. Finally (Pl. 54, C) the muscle tissue disintegrates completely, the fibrillæ, etc., are no longer visible, and the whole simulates coagulated protein material with minute granules scattered throughout. When this stage in muscular disintegration has arrived, nearly all of the other tissues have likewise disintegrated more or less and *S. disparis* may now be seen scattered everywhere. All of the pathological changes cited above are much more strikingly accentuated in artificially infected animals than in those which become infected naturally. This is to be expected, for the reason

that the number of organisms ingested in artificial infections is enormous, and consequently more toxins are elaborated, which in turn cause more striking changes. The pathology of the musculature is characteristic enough, however, so that any one can diagnose the disease from sections. On finding the muscles in the conditions illustrated by figures A and B of Plate 54, one can safely predict that *S. disparis* will be found in the alimentary tract.

At the end of this article is a detailed description of *Streptococcus disparis* with an account of the media best suited to its cultivation.

Recently Paillot [1] described a microorganism parasitic in gipsy-moth caterpillars. He named the bacterium *Diplococcus lymantriae* after the old generic name of the gipsy moth, Lymantria. The writer has carefully studied Paillot's description and finds absolutely no resemblance between his organism and *S. disparis* in either cultural or biochemical characters. Moreover, *D. lymantriae* is not very pathogenic to the caterpillars, whereas *S. disparis* is highly pathogenic.

The writer has searched the literature, but has been unable to find a description of a bacterium which in any way resembles *S. disparis*. For this reason, and especially since the organism may prove to be of some economic importance, its description as a new species seems justified.

FIELD EXPERIMENTS

During the summer of 1917 Mr. A. M. Wilcox, of this Bureau, and the writer conducted a large series of field experiments in Massachusetts in connection with *S. disparis*. One important phase of the work consisted in attempting to discover whether the organism occurred anywhere in the gipsy-moth infested territory. Efforts to find the disease in 1915 and 1916 failed, and in 1917 a much more systematic endeavor to find the organism in the field, in places where it was not artificially introduced, was unsuccessful. Hundreds of dead caterpillars from a large variety of places were collected every week and brought to the laboratory for study. The mortality was always found to be due either to parasitism by the tachina fly *Compsilura concinnata* Meigen to other insect parasites, or to wilt or some disease other than the Japanese bacterial malady. *S. disparis* was recovered from none of these localities, and therefore the statement that the new disease has not occurred in this country in the field prior to 1917 seems justified.

Another phase of the work consisted in introducing the disease by a variety of methods in woods heavily infested by the gipsy moth. The idea of the writer is not to attempt the extermination of the gipsy moth with the new bacterium, although, of course, he would wish to do so, if it were possible, but to approach the subject in the same spirit in which

[1] PAILLOT, A. MICROBES NOUVEAUX PARASITES DES CHENILLES DE LYMANTRIA DISPAR. *In* Compt. Rend. Acad. Sci. [Paris], t. 164, no. 13, p. 525–527. 1917.

the Bureau of Entomology of the United States Department of Agriculture has approached all of the other parasite-introduction work. The attempt is merely being made to introduce another foreign parasite which, if it becomes established and becomes as effective in the woods as it is in the laboratory, will prove highly beneficial. It should be added that when an epidemic of the new disease breaks out in the laboratory the percentage of mortality far surpasses the percentage of mortality in a laboratory epidemic of wilt. Every one familiar with the subject knows that wilt has been a very important factor in saving the New England forests from utter ruin. If, therefore, the matter is approached in the spirit of "parasite introduction," the economic use of insect diseases assumes a new light and should prove extremely fruitful in combating many of our noxious pests.

Contrary to expectations, the field experiments have proved highly successful in so far as the introduction and recovery of *S. disparis* is concerned. In other words, the disease has been reproduced in the field. Indeed, in two localities in Massachusetts—namely, Sherborn and North Carver—it was possible to produce quite a severe epidemic. Notwithstanding the encouraging results, however, no statistics will be given until another season has passed. A large amount of work is still needed to determine the relative importance of this method of combating the gipsy moth.

Streptococcus disparis, n. sp.

MORPHOLOGY.—From 1.5 per cent neutral nutrient agar. From 1.5 per cent neutral potato agar. From milk. From neutral nutrient bouillon.

Organism examined in these media after 24, 48, and 72 hours, after one week, and each month for eight months. No decided variations in morphology observed. Division occurred in one direction of space. Chains of 3 to 4 units frequently seen in liquid media. Diameter not 1 M. Capsulated. Motility O. Gram-positive. Stains readily. Typically a streptococcus.

NUTRIENT AGAR STROKE, 1.5 per cent.—Neutral. Growth in five days at 35° C. scanty, beaded, flat, glistening, smooth, white, opaque, odor absent, butyrous, medium unchanged.

POTATO AGAR STROKE, 1.5 per cent.—Neutral. Growth in five days at 35° C. abundant, spreading, flat, glistening, smooth, white, opaque, odor absent, butyrous, medium unchanged.

POTATO.—Growth moderate, spreading, flat, odor absent, butyrous, color of medium unchanged.

GELATIN STAB.—Growth best at top. Line of puncture beaded. No liquefaction. Medium unchanged.

NUTRIENT BROTH.—No ring, no pellicle, clouding slight, clearing after 15 days, slight sediment, odor absent.

MILK.—Coagulation delayed. Extrusion of whey. Color of medium unchanged. No peptonization.

LITMUS MILK.—Acid, prompt reduction, coagulation delayed, extrusion of whey, no peptonization.

DUNHAM'S PEPTONE SOLUTION.—Clouding very slight. Growth poor.

GELATIN COLONIES.—Growth slow, colonies very small and majority under surface. Surface colonies round, slightly convex, edge entire, no liquefaction.

NUTRIENT AGAR COLONIES.—Growth slow. Majority of colonies under surface and oblong. Surface colonies round, smooth, convex, edge entire, internal structure finely granular. Diameter 0.25 to 0.33 mm.

POTATO AGAR COLONIES.—Growth rapid. Majority of colonies under surface and oblong. Surface colonies round, smooth, convex, edge entire, internal structure finely granular. Diameter 1 to 1.5 mm.

NH_3 PRODUCTION.—Absent.

NITRATE SOLUTION.—Nitrates not reduced.

INDOL PRODUCTION.—Absent.

HYDROGEN SULPHID PRODUCTION.—Absent.

FERMENTATION OF CARBOHYDRATES WITH FORMATION OF ACID AND GAS.—

	Gas.	Acid.
Dextrose	o	+
Levulose	o	+
Saccharose	o	+
Maltose	o	+
Lactose	o	+
Mannit	o	+
Adonit	o	+
Dulcit	o	+

OXYGEN REQUIREMENTS.—Facultative anerobe.

BEST MEDIA FOR CULTIVATION.—Solid: 1.5 per cent, neutral potato agar. Liquid: Neutral nutrient bouillon containing a carbohydrate, especially bouillon containing about 1 per cent of saccharose, maltose, or mannit.

PATHOGENICITY.—Pathogenic to the caterpillars of the American, European, and Japanese races of the gipsy moth (*Porthetria dispar* Linnaeus). Not pathogenic to silkworms (*Bombyx mori* Linnaeus) and army worms (*Cirphis unipuncta* Haworth) when fed *per os*. Guinea pigs, rabbits, and human beings when fed pure culture *per os* not affected.

SUMMARY

(1) In 1915 a new infectious disease was found in certain cultures of the Japanese race of the gipsy moth.

(2) The infection spread later to cultures of the American race.

(3) The disease is clinically, pathologically, and etiologically distinct from wilt.

(4) A streptococcus was found to be the causative agent.

(5) The bacterium is new to science and is here described under the name "*Streptococcus disparis*."

(6) During the early stages of the disease the bacterium is found throughout the alimentary tract of the gipsy-moth caterpillars.

(7) During the later stages of the disease and after death the bacterium invades practically all the tissues.

(8) *S. disparis* invades the alimentary tract with the ingested food.

(9) Healthy animals naturally become infected by eating food soiled by the feces of infected animals.

(10) *S. disparis* is not pathogenic to silkworms (*Bombyx mori* Linnaeus) or to army worms (*Cirphis unipuncta* Haworth).

(11) *S. disparis* is not pathogenic to human beings, guinea pigs, or rabbits.

(12) The most striking pathological changes during the course of the disease occur in the muscle tissues of the caterpillar.

(13) Many observations and tests show that the new disease did not occur in this country prior to 1917.

(14) Field experiments were conducted with *S. disparis* in sections of the gipsy-moth infested territory.

(15) Success was obtained many times in reproducing the disease in the field.

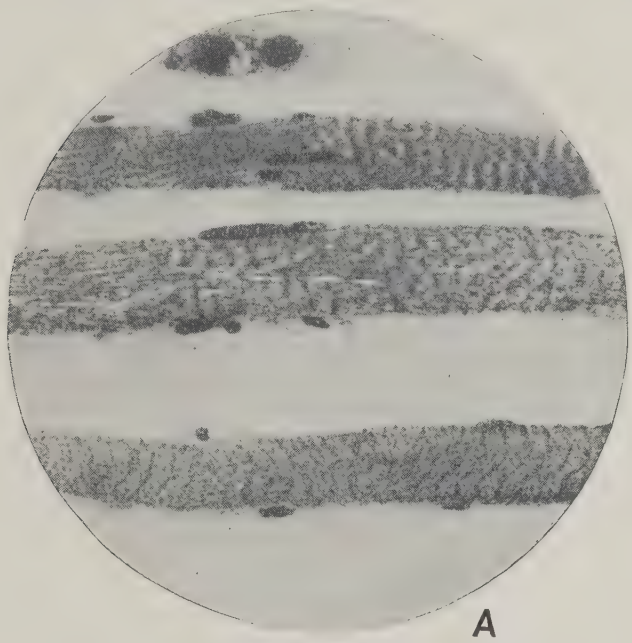
(16) In two places quite a severe epidemic was created.

PLATE 54

A.—Photomicrograph of normal and early pathological gipsy-moth muscle tissue. × 540.

B.—Photomicrograph of late pathological gipsy-moth muscle tissue showing separation of fibrillæ. × 540.

C.—Photomicrograph of last stage in pathology of gipsy-moth muscle tissue, showing complete disintegration. × 540.



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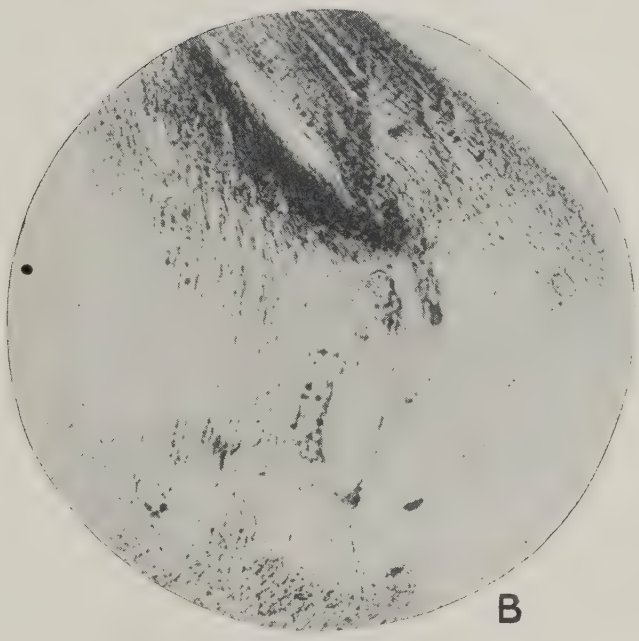

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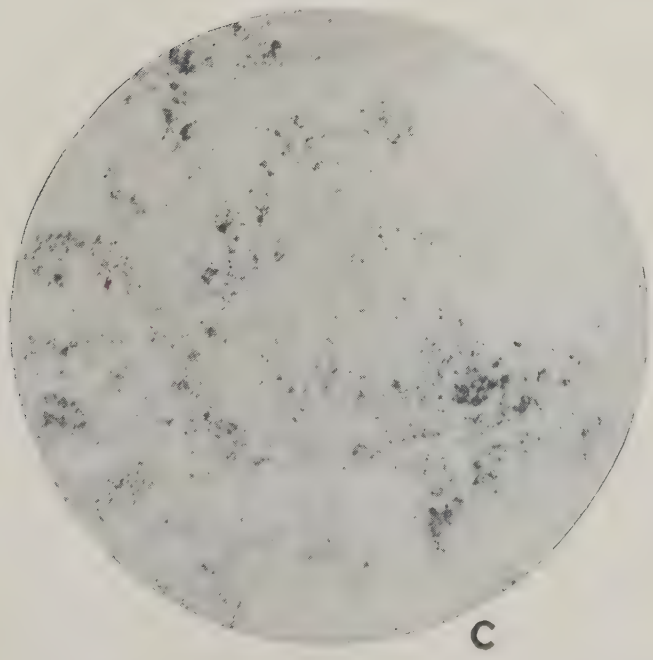

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PHYSICAL PROPERTIES GOVERNING THE EFFICACY OF CONTACT INSECTICIDES[1]

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INTRODUCTION

It was considered by Shafer (*16*)[2] that the vapor of contact insecticides such as kerosene, gasoline, creolin, and pyrethrum were responsible for the death of insects to which these materials were applied. It was therefore assumed as a working basis that the volatility of organic compounds, which has previously been shown to be an index of the toxicity of their vapors to insects (*11*, *12*), would also be an index of the toxicity of these compounds when used as contact sprays. In working with insect eggs (*14*), however, it was found that materials not sufficiently volatile to kill insects or their eggs by their vapor within a reasonable length of time, were among the most effective materials when applied to the eggs as liquids. Further studies in which different fractions of kerosene were used (*13*) revealed the fact that the least volatile fractions were the most effective as contact insecticides, while they failed to kill insects which were exposed only to their vapor.

With these results in mind it was considered advisable to determine the physical properties governing the entrance into the insect of a contact insecticide, and wherein this differs from the penetration of the vapor.

WETTING AND SPREADING OF THE INSECTICIDE

It is common observation that when some contact insecticides strike an insect they form into round droplets which roll off the body, while others spread out, forming a film over the insect. This phenomenon of the spreading out of the insecticide over the body has been often termed "wetting" or "spreading" and is often confused with the wetting and spreading of the insecticide over the surface of the leaves sprayed, the terms "wetting" and "spreading" being used synonymously. Vermorel and Dantony (*18*, *19*, *20*), Lefroy (*5*), and more recently Cooper and

[1] Published, with the approval of the Director, as Paper 112 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited." p. 537–538.

Nuttall (2) have studied the physical principles governing the wetting and spreading of contact insecticides and have endeavored to devise means by which these important properties may be easily measured. In all of these papers the authors have failed to distinguish between wetting and spreading. In this paper a distinction will be drawn between these two terms. If a liquid is placed upon a solid, and there is a specific attraction between the two, they will come into actual contact. The slight chemical affinity exhibited between the two substances is what is denoted as wetting, or adhesion between the liquid and the solid. For example, if a drop of mercury is placed upon glass, there is no specific attraction between the two, owing to the film of moisture and air on the surface of the glass (*3, p. 176*). Hence, the mercury is said not to wet the glass, and there is no adhesion between the two. By boiling the mercury in a glass tube and thus expelling the moisture and air between the mercury and the glass, it is found that there is an actual wetting of the glass by the mercury, as is indicated by adhesion.

If a liquid is brought into contact with a solid and wetting takes place, the spreading of the drop into a thin film may or may not occur. The law governing spreading has been carefully explained by Cooper and Nuttall (2). They find that if the surface tension of the substance upon which the spray is placed is greater than the surface tension of the spray plus the surface tension at their interface (interfacial tension), the liquid will spread. Otherwise there will be no spreading. Bigelow and Hunter (1) have given a very much simpler explanation of the whole matter. They consider (*p. 377–387*) that if a liquid is in contact with a solid—that is, actually wetting the solid—two forces are at work:

> First, the cohesion between the like particles of the liquid which, in the surface layer is denoted by the phrase "surface tension," and second, the adhesion between the liquid and the walls [solid].

Thus, if adhesion to the solid is stronger than the cohesion of the liquid, the liquid will spread over the solid. The same law applies when two liquids are in contact. The following experiment will serve as an example. If a filter paper is soaked in water and spread out flat on a glass plate, a drop of kerosene on this wet paper will quickly spread into a thin film. In this case the adhesion between the water and the kerosene is greater than the cohesion of the kerosene. On the other hand, if the filter paper is soaked in kerosene and a drop of water placed upon it, the water does not spread out into a thin layer. In this case the cohesion of the water is greater than the adhesion between the water and the kerosene, which is the same in both experiments. Thus, it is clear that there must be wetting before there can be spreading, but it does not necessarily follow that when there is wetting there must be spreading, for otherwise the water would have spread over the kerosene.

One other factor has a considerable influence on the spreading of an insecticide—namely, viscosity. Viscosity may be defined as "the inter-

nal friction of a liquid." Although the viscosity does not influence the ultimate extent to which a liquid may spread, it does have a very decided influence on the rate of spread. It is possible that a liquid may be so viscous that the rate of spread may be reduced to such an extent as to make it valueless as an insecticide.

From the foregoing statements it is apparent that when a spray strikes an insect, if there is a chemical affinity between the insecticide and the chitin which forms the outer covering of the insect, wetting will take place and the insecticide will adhere. If the cohesion of the spray is less than the adhesion between the chitin and the spray, then the liquid will spread over the body of the insect. The rate at which this spreading will take place is governed by the viscosity of the liquid. If the cohesion is greater than adhesion, the spray will form into droplets which tend to roll off. The same result is obtained when the spray does not wet the insect. It is apparent therefore that in a contact insecticide it is important not only that the liquid should wet the chitin, but also that the adhesion of the liquid to the chitin should be greater than the cohesion of the liquid.

RELATION BETWEEN SPREADING AND CAPILLARITY

The rise of a liquid in a capillary tube is governed by the same laws as the spreading of a liquid over the surface of a solid. First, unless the liquid has a specific attraction for the material of which the tube is composed (wetting), there can be no capillary rise. Second, unless the adhesion between the liquid and the walls of the tube is greater than the cohesion of the liquid, there can be no capillary rise. This is well shown by Bigelow and Hunter's (*1*) studies of the rise of water in capillary tubes of different materials. In their experiments the cohesion of water remained the same, but owing to different degrees of adhesion between the water and the walls of the capillary tubes, variations were noted in the height to which the liquid rose in tubes of different materials. It is evident, therefore, that since the tracheæ are lined with chitin, similar to the covering of the body wall, insecticides which will spread over the body will also penetrate the tracheæ by capillarity, while those insecticides which do not spread over the insect, even though they may come in contact with a spiracle, will not be able to penetrate the tracheæ.

Contact insecticides may therefore be divided roughly into two groups: First, those which wet the insect and, owing to greater adhesion than cohesion, are able to spread over the surface of the body and pass up the tracheæ by capillarity. Second, those which wet the insect, but which, owing to a higher cohesion than adhesion, are able neither to spread over the surface nor to gain entrance into the tracheæ by capillarity.

PENETRATION OF LIQUIDS INTO THE TRACHEÆ

From the results of Cooper and Nuttall (2) it would appear necessary to make a determination of the surface tension of the insecticide, the surface tension of the chitin, and the surface tension at the interface of the chitin and the insecticide before it could be determined whether or not the liquid would spread over the body and penetrate the tracheæ. From the results of Bigelow and Hunter (1), however, the capillary rise in a tube really determines whether the adhesion between the liquid and the solid is greater than the cohesion of the liquid, or, in other words, is a

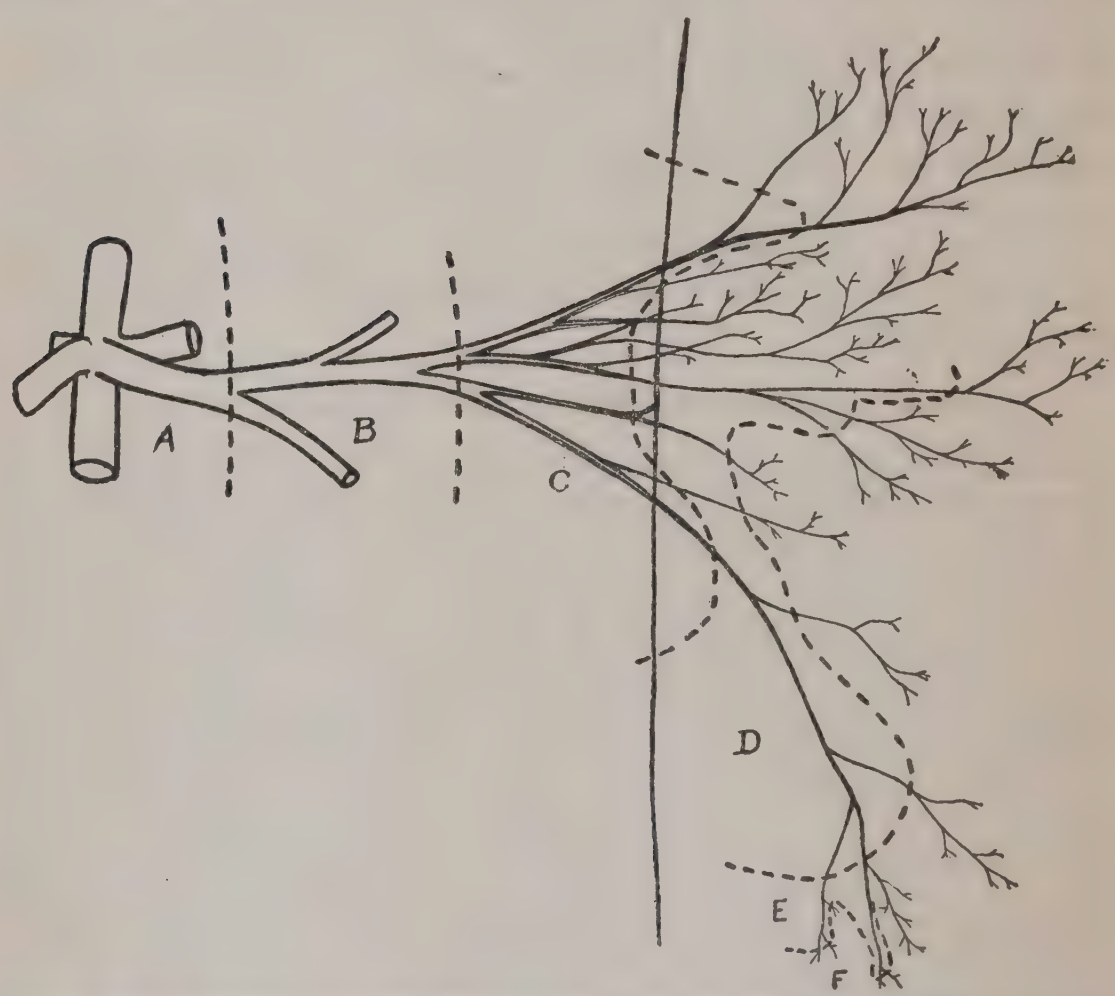

FIG. 1.—Sketch of a trachea of the cockroach divided into sections A, B, C, etc., to indicate the distance the various oils penetrated. See Table I.

means of determining whether the surface tension of the solid is greater than the surface tension at the interface between the solid and the liquid plus the surface tension of the liquid. By placing an insect in the insecticide to be studied for a short period and then dissecting it, it is possible to determine whether or not the insecticide has penetrated the tracheæ, and approximately how far. These results give an index of the spreading ability of the different materials.

The cockroach (*Blatella germanica* L.) was used in the following experiments for determining the penetration of insecticides into the tracheæ. The results were checked over with certain of the compounds using the wax-moth larvæ (*Galleria mellonella*), the larvæ of the Indian meal moth

(*Plodia interpunctella* Hbn.), and certain aphids. The methods of procedure were as follows: The liquid to be tested was stained with Sudan III or trypan-blue, both of which are colloids, and therefore will not pass through a semipermeable membrane. The cockroach was placed into the liquid and allowed to remain for from 15 minutes to 2 hours, depending upon the viscosity of the liquid. After a sufficient time had been allowed for the liquid to penetrate, the cockroach was removed and opened on the ventral side. The tracheæ which were penetrated by the material stood out either red or blue, and it was very easy to determine the extent of the penetration.

Table I gives a list of the materials used and shows their power of penetration. The letters represent the distance the material penetrated into the tracheæ as illustrated in figure 1.

TABLE I.—*Materials used in experimental work, showing their power of penetration*

Name.	Penetration.[a]	Name.	Penetration.[a]
Distilled water	O	Lubricating oil 6	D to E
Saponin solution (1:500)	O	Cod-liver oil	D to E
Gelatin solution (1:1,000)	O	Oil of pine needles	E
Nicotine solution	O	Oil of juniper	E
Glycerin	O	Rape-seed oil	E
Lime-sulphur solution	O	Raw linseed oil	E
Alcohol (50 per cent)	O	Lubricating oil 1	E
Milk	O	Peanut oil	E
Ivory soap (1:150 at 24° C.)	A	Knochen oil	E
Croton oil	A	Lubricating oil 2	E
Lubricating oil 11	A	Wood creosote	E
Nicotine	A	Olive oil	E
Acetic acid	A	Acetone	E
Furfurol	A to B	Ether	E
Soft soap (1:150 at 24° C.)	B	Gasoline	E
Yellow soap (1:150 at 24° C.)	B	Benzyl alcohol	E
Ivory soap (1:150 at 36° C.)	B	Chlorbenzene	E
Oil of tar	B	Nitroxylene	E
Cassia oil	B	Oil of verbena	E to F
Clove oil	B	Oil of camphor	E to F
Lubricating oil 7	B	Oil of lemon	E to F
Boiled linseed oil	B	Oil of citronella	E to F
Lard oil	B	Oil of lavender	F
Castor oil	B	Oil of sassafras	F
Absolute alcohol	B	Oil of eucalyptus	F
Trimethylene cyanid	B	Oil of bergamot	F
Nitrobenzene	B	Oil of hemlock	F
Methyl salicylate	B	Oil of tansy	F
Quinolin	B to C	Oil of mustard	F
Oleoresin of black pepper	C	Red oil	F
Castile soap (1:150 at 24° C.)	C	Coal-tar creosote	F
Cedar oil	C	Cottonseed oil	F
Fish oil	C	Oil of turpentine	F
Petrolatum	C	Carbon bisulphid	F
Amyl alcohol	C	Xylene	F
Oil of bitter almonds	C to D	Chloroform	F
Whale oil	D	Chlorpicrin	F
Eugenol	D	Petroleum ether	F
Lubricating oil 5	D to E		

[a] See figure 1 for explanation of letters.

It will be noticed that aqueous solutions other than soap solutions do not penetrate the tracheæ. It was interesting to note that both nicotine and absolute alcohol are able to penetrate the tracheæ, but when greatly diluted with water they are no longer able to enter. Those substances which in an aqueous solution exhibit surface viscosity, such as saponin, gelatin, and casein, do not penetrate the tracheæ. Those materials have frequently been employed in preparing emulsions (*4*, *20*, *21*). It is apparent from the table as a whole that compounds which are soluble in ether or are capable of dissolving fats or oils are able to spread over the body of the cockroach and penetrate the tracheæ. Such substances as acetic acid, furfurol, and nicotine are not particularly good fat solvents, and it may be noted that they did not penetrate any great distance into the tracheæ. Compounds with a high viscosity, such as lubricating oil, failed to penetrate far into the tracheæ; but if these viscid substances had been given a longer time they would undoubtedly have penetrated much farther. Some soap solutions, such as ivory, gel at room temperatures in dilutions ordinarily used in spraying (1:150 or 200), and are therefore unable to penetrate to any great extent. The penetration of such a soap solution is increased when its cohesion is reduced by raising the temperature of the solution. Soft soap and yellow soap were liquid at the same dilution and penetrated better than Ivory soap, while Castile soap, manufactured from the liquid oleic acid, penetrates very well.

According to Morgulis (*15*) the exact chemical composition of chitin is still questionable. From our results its composition must be such that it is easily wetted by oils and oil solvents, or perhaps the surface of the chitin itself contains or is coated with an oily or a fatty substance. These results can not be applied to all insects, as some have special coverings of wax over the chitin. The spreading of sprays on such insects can, however, be determined by our methods with little trouble.

RELATION OF VISCOSITY AND VOLATILITY TO THE PENETRATION OF THE TRACHEÆ

The foregoing results have been primarily concerned with the pure materials. Inasmuch as the pure materials are seldom applied in actual practice, the question arises as to what takes place, when an emulsion of an oil is used in spraying. By placing drops of the emulsion on the wings, and also on the bodies of cockroaches, it was found that the oil droplets were completely surrounded by the emulsifier and did not come in contact with the body of the insect. The ability of such a spray to adhere to the insect, to spread over the body, and to penetrate the tracheæ depends, therefore, on the character of the emulsifier and not on the emulsified oil. Three distinct types of emulsions were noted: First, emulsions made with gelatin or saponin formed a round drop and tended to roll off the insect, just as did pure aqueous solutions of these

materials. Second, emulsions made with Castile or soft soap adhered to the insects and spread over the body, penetrated the tracheæ, carrying the emulsified oil with them. Third, emulsions made with using Ivory soap adhered to the body of the insect; but, owing to the high cohesion of the liquid, spread very slowly. In the third case it was noticed that in a comparatively short time, depending upon the temperature and humidity of the surrounding atmosphere, the water evaporated and the emulsion broke down, after which the oil spread over the surface of the body, penetrating the tracheæ. The length of time required for the breaking of the emulsion was from 5 to 30 minutes. If the oil was rather volatile, it would evaporate before it succeeded in penetrating the tracheæ. By spraying a number of insects it was determined that oils more volatile than xylene were too volatile to succeed in penetrating the tracheæ in large enough quantities to result in the death of the insect. On the other hand, the oil may be so viscous that, even after the breaking of the emulsion, it is unable to spread over the body and enter the tracheæ in a reasonable length of time. The viscosity of a number of oils was determined in terms of water by measuring the length of time required for 5 c. c. of the liquid to flow through a glass tube of small diameter arranged in the form of a stalagmometer. The results of these are shown in Table II.

By placing a small drop of oil on the wing of a cochroach and watching it under the miscroscope it was possible to divide the oils into four classes: (1) Those spreading rapidly, (2) those spreading slowly, (3) those spreading very slowly, and (4) those spreading so slowly as to preclude any possibility of their reaching the spiracles in a reasonable length of time.

The oils in Table II are divided into these four groups. It is apparent that oils with a viscosity as high or higher than castor oil are so viscous that their value as contact insecticides is questionable.

TABLE II.—*Viscosity of various oils*

Material.	Time.	Viscosity, in terms of water.	Spreading ability.
	Seconds.		
Water (distilled)	3.3	1.00	
Kerosene (b. p. 140°–187°)	3.6	1.10	Rapid spreader.
Kerosene, unfractionated	4.6	1.40	Do.
Kerosene (234°–280°)	5.2	1.57	Do.
Turpentine oil	5.4	1.60	Do.
Lubricating oil 1	46.5	14.10	Slow spreader.
Red oil	54.0	16.32	Do.
Fish oil	66.0	20.00	Do.
Lubricating oil 2	68.0	20.60	Do.
Whale oil	73.0	22.12	Do.
Boiled linseed oil	77.0	23.33	Do.
Raw linseed oil	81.0	24.54	Do.
Cottonseed oil	88.0	26.66	Do.
Lubricating oil 3	99.0	30.00	Do.
Lard oil	103.0	31.51	Do.
Olive oil	104.0	31.82	Do.
Lubricating oil 4	115.0	34.85	Do.
Knochen oil	128.0	35.88	Do.
Lubricating oil 5	177.0	53.66	Do.
Lubricating oil 6	194.0	58.78	Do.
Lubricating oil 7	495.0	150.00	Do.
Castor oil	1,205.0	365.15	Very slow spreader.
Lubricating oil 8	1,472.0	446.06	Do.
Lubricating oil 9	1,740.0	530.30	Do.
Lubricating oil 10	2,340.0	709.09	Do.
Lubricating oil 11	2,610.0	790.90	Do.
Lubricating oil 12	2,707.0	820.90	Do.
Lubricating oil 13	3,568.0	1,081.30	Too slow for effective work.
Lubricating oil 14	5,462.0	1,655.15	Do.
Lubricating oil 15	7,835.0	2,374.27	Do.

PENETRATION OF THE INSECTICIDE INTO THE TISSUES

Shafer (*16*) has shown that contact insecticides, such as kerosene and others of a similar nature, are able to penetrate the tracheæ of an insect, but he considers that it is the vapor from these substances, which is responsible for the killing, inasmuch as the rate at which the liquid itself will pass through the chitin is too slow to account for the death of the insect. He dissolved Sudan III in kerosene to show the passage of the oil through the walls of the tracheæ. At the time of the death of the insect he could find no evidence of a red stain in the tissues, and it was not until the insect had been dead for a long time and the fat bodies had been partially dissolved that he could detect traces of the stain in the tissues. Sudan III, however, is a colloid, and would not be able to pass but very slowly through a semipermeable membrane such as chitin. Thus, the inability of the stain to pass through the chitin did not necessarily imply that the kerosene had not been able to penetrate into the tissues. If the experiment is repeated with picric acid instead of Sudan III, it is found that the tissues are very quickly stained yellow. There are, however, a number of objections to the use of a stain to indicate the

passage of a liquid, as it is quite possible that the stain could penetrate by being absorbed by the walls of the tracheæ without any penetration of the oil. If a few pieces of chitin are placed in kerosene stained with picric acid, they are capable of absorbing practically all the stain from the oil. On the other hand, it is quite possible that the amount of the insecticide which might penetrate and kill the insect would be so small that the amount of stain carried with it could not be detected. Further, it would be impossible to determine the penetration of a vapor by a stain in the insecticide. Chemical tests for insecticides in the tissues in many cases would not be delicate enough for the certain detection of their presence or absence. Finally, after a number of trials, it was found that the best method was to use an indicator for dead tissues. Trypan blue is a water-soluble colloid which does not penetrate the living tissues, but is able to penetrate and stain dead tissues. This was selected as the most suitable indicator. Larvæ of the wax moth were used in these experiments. If a living, untreated larva is opened and the tissues covered with an aqueous solution of trypan blue for a period of two minutes and the stain then removed and the larva examined under water, it will be found that there are particles of the stain adhering along the midintestine, more or less on the silk glands, and along the nerve cord. This does not seem to be a true staining of the tissues, but rather the adherence of particles of the stain, which are difficult to remove by washing (adsorptions).

When an insect has been treated with an effective contact insecticide and opened and stained just before it dies, the tissues which have been killed are stained a deep blue, thus indicating the point of entrance of the poison. No effort was made in this work to determine which tissues were primarily effected by the chemicals. As a counter stain, to show to what extent the materials penetrated the tracheæ, Sudan III was used with the oils and resin with the aqueous solutions. The penetration into the insect of the different insecticides are given in Table III.

TABLE III.—*Penetration of materials as indicated by Sudan III, with trypan-blue method*

FUMIGATION VAPORS

Name.	Time.	Effect.
Nitrobenzene	5½ hours	Blue spots in flat tracheæ to digestive tract only where condensation occurs. Blue along fine tracheæ.
Nicotine	3 hours	Do.
Chlorpicrin	do	Do.
Carbon bisulphid	6½ hours	Blue spots along large and fine tracheæ. No condensation noted.

TABLE III.—*Penetration of materials as indicated by Sudan III, with trypan-blue method*—Continued

Name.	Time.	Effect.
NONWETTING SOLUTIONS		
Nicotine solution	1½ hours	Fine tracheæ stained blue. Condensation not noted, but probably present.
Nicotine sulphate	7½ hours	Tissues along large and fine tracheæ stained and blue condensation in flat tracheæ to digestive tract.
Furfurol solution	17 hours	Blue patches on flat tracheæ to digestive tract. Very slight blue along fine tracheæ. Condensation evidently responsible for stains in large tracheæ.
SOAP SOLUTIONS		
Ivory (1 : 800)	31 hours	Blue stain along some of the large tracheæ where red-stained soap solution penetrated.
Soft (1 : 300)	3½ hours	Blue stains along walls of red-stained tracheæ.
VERY VOLATILE OILS AND ACIDS		
Acetic acid	1 hour	Blue spots on large tracheæ and along fine tracheæ. Red-stained and penetrated body wall in thoracic region.
Chlorpicrin	10 minutes	Large and fine tracheæ stained blue. Condensation in large flat tracheæ to digestive tract.
Chloroform	20 minutes	Blue along red-stained tracheæ. Vapor going ahead of red-stained material has little influence. More effect if treated longer.
Gasoline	30 minutes	Passed through tracheæ and partially dissolved fat bodies, but caused little bluing.
VOLATILE OILS AND ACIDS		
Butyric acid	40 minutes	Blue stain along all particularly fine tracheæ. Red stain penetrated body wall as in acetic acid.
Wood creosote	30 minutes	Blue stain along red-stained tracheæ, particularly those to digestive tract.
Kerosene (230°–280°)	2 hours	Blue stain along red-stained tracheæ, particularly those to digestive tract. Blue along some fine tracheæ.
Chlorbenzene	.....	Blue along red-stained tracheæ. No blue or very little beyond red stain.

TABLE III.—*Penetration of materials as indicated by Sudan III, with trypan-blue method*—Continued

ESSENTIAL OILS

Name.	Time.	Effect.
Tar oil	5 hours	Blue along tracheæ to which red penetrated. Very slight blue stain beyond red.
Eucalyptus oil	1½ hours	Blue along red-stained tracheæ but not beyond.
Sassafras oil	2 hours	Blue beyond point of tracheæ to which red penetrated and in a few places along red-stained tracheæ.
Turpentine oil	½ to 1 hour	Red passed through fine tracheæ and into fat bodies. Blue along tracheæ. Red through body wall between segments.

FIXED OILS AND THEIR ACIDS

Paraffin oil	5 to 20 hours	Blue along red-stained tracheæ, but not beyond.
Olive oil	do	Do.
Oleic acid	do	Do.
Peanut oil	do	Do.
Croton oil	do	Do.
Cottonseed oil	do	Do.
Rape-seed oil	do	Do.

LUBRICATING OILS

Lubricating oil 6	5 to 20 hours	Slight blue stain along red-stained tracheæ.
Lubricating oil 1	do	Do.

The insecticides may be divided into four different groups: (1) The nonvolatile insecticides which as liquids penetrate the tracheæ. (2) The volatile insecticides which are able to penetrate the tracheæ as liquids. (3) The volatile insecticides which only penetrate the tracheæ in vapor form. (4) The nonvolatile insecticides which decompose on contact with the insect, producing a vapor which is capable of entering the tracheæ. The results in Table III show that relatively nonvolatile oils pass through the walls of the tracheæ only where the liquid has penetrated.

Soap solutions are particularly interesting, since when a soap is dissolved in water it is hydrolyzed, some of the soap molecules reacting with the water to form sodium hydrate and the free fatty acid. This free fatty acid unites with the fatty acid of that portion of the soap which has not been hydrolyzed, forming a sol (*10*). Since the chitin is a semipermeable membrane, the sol is able to pass through but very slowly into the body of the insect. The sodium hydrate in the solution is no doubt the portion which accounts for death. Experimental

evidence shows that solutions of soaps containing a large percentage of free alkali are more toxic than those which are practically neutral.

Volatile oils and acids show a blue-staining along the walls of the tracheæ where the liquid has penetrated, and also blue blotches along the walls beyond this point. Examination of insects which have been treated for varying lengths of time show that the blue blotches caused by the vapor of the chemical appear more quickly than the blue caused by the penetration of the liquid itself, showing that the vapor is able to penetrate the walls of the tracheæ more quickly than the liquid. It might be noted here that these results are somewhat modified by the fact that the vapor is passing through somewhat thinner chitin than that through which the liquid must pass.

Aqueous solutions such as nicotine do not penetrate the tracheæ other than in the form of a vapor. The blue in this case occurs in blotches along the walls of the tracheæ. What was evidently a condensation of the vapor appeared in the larger tracheæ and the blue staining of the tissues was particularly strong at these points. This confirms the observations of McIndoo (*8*), who has shown by chemical means the condensation of nicotine within the tracheal tubes. When nicotine sulphate comes in contact with the body of the insect, it is slowly decomposed, with the formation of nicotine which enters as a vapor. Such a decomposition is no doubt the explanation of the results of Lovett (*6*), who found that leaves sprayed with nicotine sulphate even when dry are repellent and poisonous to insect larvæ, even though not taken internally.

To such aqueous solutions, which normally are not able to enter the tracheæ, the addition of soap increases their efficiency, as it enables the liquid to spread over the body and enter the spiracles. The addition of too much soap will somewhat decrease the efficiency of the spray, owing to increased cohesion. Some of the results obtained in the use of nicotine sulphate and fish-oil-soap sprays by Smith (*17*) are thus explained.

In general there was little evidence of penetration of the insecticides through the body wall. This does not necessarily mean that the compounds are not able to penetrate the chitin, but that they were unable to do so prior to their entrance by way of the tracheæ. Compounds which are readily soluble in water and readily diffuse in aqueous solutions were frequently found to have gained entrance through the anus and through the mouth. Alcohol, acetic acid, and sometimes soap showed such penetration. The more viscous compounds require very much longer to enter the insect than the compounds with a low viscosity. Lime-sulphur differs from other contact insecticides in its action, but its action has been fully described by Shafer (*16*).

PENETRATION OF FUMIGANTS

As will be noted in Table III, fumigants gain entrance into the insect by way of the tracheæ. In many cases what appeared as a condensation was noticed within the tracheæ and in such cases blue staining, indicating

dead tissues, appeared at these points. These results explain why the volatility of organic compounds should be related to their toxicity (*12*). The more nearly the atmosphere is saturated with the vapor, the more likelihood there is of a condensation in the tracheæ. Even though there is no condensation, the same forces are at work. One of these forces is the tendency for the vapor to condense on coming in contact with the chitinous walls of the tracheæ. The other force is the tendency of the compound to reevaporate from the tracheal walls. In the least volatile this tendency to reevaporate is generally diminished, while in the more volatile compounds, in order to reduce this tendency to reevaporate, very large quantities of the chemical must be present in the air. It is thus apparent that an insect may be killed with comparatively small doses of slightly volatile compounds, while it may require a much heavier dose of a more volatile material. The volatility of organic compounds is therefore, in general, an index of their ability to penetrate into the body of the insect, and inasmuch as the compound which can not penetrate will be unable to kill, it is apparent that the volatility is correlated with the toxicity. One notable exception mentioned in a previous paper (*12*) is that of chlorpicrin, which in very minute quantities is able to kill the insect. In our experiments in tracing the penetration of fumigants it was noticed that this material was able to penetrate the walls of the tracheæ and kill the insect very quickly. This may be due to one of two factors: First, the extreme toxicity of chlorpicrin, or second, an abnormal power of penetration.

The following experiment throws light on this question. Acetic acid and benzene are of about the same volatility as chlorpicrin. On assuming, therefore, for the sake of the experiment, that their powers of penetration are equal, it was determined to test their comparative toxicity to insect tissues. Three living wax-moth larvæ were opened on the ventral side and spread out. One was treated directly with acetic acid, the second with benzene, and the third with chlorpicrin for a period of one-half minute. The chemicals were then quickly removed, and the tissues were washed with water and then treated with trypan blue. The larva treated with acetic acid showed a very intense blue-staining throughout the tissues. The larva treated with benzene showed but slight staining. The larva treated with chlorpicrin showed more staining than benzene but much less than that of acetic acid. Of the three compounds acetic acid was by far the most poisonous to the tissues. In actual fumigation, dipping, or spraying, the death of the insect occurs most quickly with chlorpicrin. This material, therefore, must owe its abnormal toxicity to its ability to be absorbed by the chitin and passed into the body. The reason for this high power of penetration of chitin by chlorpicrin will be the object of further study.

SUMMARY

From the general results reported in this paper it appears that the physical properties as well as the chemical properties have an important bearing upon the efficiency of the contact spray. Even though the spray may contain a very active poison, it will not be effective unless it conforms to certain physical requirements—that is, the ability to vaporize and penetrate in the form of a vapor or to spread over the insect and penetrate in the liquid form. The results reported by McClintock, Houghton, and Hamilton (7) show very clearly that this is true. The results in the use of quassia with or without soap as reported by McIndoo and Sievers (9) are another example, and it is a common observation that the addition of soap to nicotine sprays increases their efficiency. The following are some of the principles which must be kept in mind in studying the effects of contact insecticides.

(1) Contact insecticides may be divided into two groups: (a) Those which spread over the body of the insect and penetrate the tracheæ. (b) Those which are not able to spread over the insect and do not penetrate the tracheæ.

(2) Contact insecticides which are either soluble in ether or chloroform or are fat solvents are able to spread over the insect and enter the tracheæ.

(3) The rate of spread of these insecticides is governed by their viscosity and cohesion.

(4) Compounds with a viscosity as high or higher than castor oil spread so slowly that in general they may be classed as poor insecticides.

(5) Compounds more volatile than xylene evaporate too quickly for effective work.

(6) Sprays in the form of emulsions may enter the tracheæ as such, or the oil remaining after the emulsions is broken down may spread over the insect and enter the spiracles.

(7) Relatively nonvolatile oils penetrate the body of the insect directly through the walls of the tracheæ as liquids, the rate depending upon the viscosity.

(8) Volatile oils may penetrate the walls of the tracheæ in either vapor or liquid form.

(9) Sprays which are unable to enter the tracheæ in liquid form may penetrate and pass through the tracheal walls as vapor.

(10) Fumigants gain entrance and pass through the tracheal walls in vapor form.

(11) Slightly volatile compounds tend to condense upon the tracheal walls, owing to the fact that small quantities are sufficient to saturate the atmosphere. Owing to this high saturation, these condensations tend to penetrate the chitin rather than to reevaporate. Volatility is an index of the ability of the compound to gain entrance into the insect and is therefore closely correlated wiith toxicity.

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INOCULATION EXPERIMENTS WITH SPECIES OF COCCOMYCES FROM STONE FRUITS[1]

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INTRODUCTION

For three years the writer[2] has had under investigation leafspot diseases of cherries and plums (*Prunus* spp.) in Wisconsin. In connection with this work in 1916 and 1917, more than 1,000 cross-inoculation experiments were conducted with *Coccomyces* spp. from a number of the more common wild and cultivated species of Prunus of the State, with the aim of furthering our understanding of the relationships of these pathogenes to their hosts and to one another. This paper is a report of progress on these experiments. It is presented at this juncture because of the temporary discontinuance of the work owing to the stress of war conditions. Final conclusions and the discussion of results in relation to the work of others and in their bearing upon other phases of the leafspot problem are reserved for a future paper.

EXPERIMENTS OF 1916

Preliminary experiments in 1915 and in the spring of 1916, conducted in the light of the results of the similar work of Higgins,[3] formed a basis of experience upon which to develop methods for further work. These tests showed that satisfactory experiments might be conducted either in the greenhouse or out of doors, and that, particularly in the outdoor inoculations, it is essential to have means of maintaining high humidities about the experimental plants or organs without the development of excessively high temperatures. In order that the conditions of the experiments might be as nearly normal as possible, the further inoculations of 1916 were made out of doors. The following method was employed, especial attention being given to standardizaation of technic, with the aim of increasing both the comparative value and the facility of presentation of results.

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TECHNIC OF INOCULATIONS

EXPERIMENTAL PLANTS.[1]—In each experiment, the following plants were used: *Prunus cerasus* L. (Montmorency Stark), *P. avium* L. A (Windsor), *P. avium* B (seedling), *P. mahaleb* L., *P. pennsylvanica* L. f., *P. serotina* Ehrh., *P. padus* L., *P. virginiana* L., *P. domestica* L. (Lombard), *P. insititia* L. (Shropshire), *P. americana* Marsh. A (De-Soto), *P. americana* B, *P. salicina* Lindl. (Burbank), *P. munsoniana* Wight and Hedrick (Wild Goose), *Amygdalus persica* L. (Elberta), *A. persica nectarina* Ait. (Boston), *P. armeniaca* L. (Alexander), and *P. besseyi* Bailey.

Except in cases where varietal names are given, the experimental plants were seedlings or cuttings. They were 1-to-2-year-old trees obtained, so far as feasible, from reliable nurseries in the upper Mississippi Valley. *P. americana* and *P. virginiana* were collected in the vicinity of Madison. The identification of all species was confirmed. The trees were assembled in the spring and heeled in until the middle of May, when they were severely pruned and set in the pathological garden in such fashion that suitable groups might be covered by moist chambers. Each group consisted of two rows, 14 inches apart, in which the plants were set at 10-inch intervals, except near the middle, where a 3-foot space was left in order to facilitate the manipulation of the moist compartments. Each plot contained one tree of each experimental species or variety, and in all plots the plants were grouped in the same order. The plots were disposed at intervals of 12 feet in the direction of the rows, and 6 feet laterally. Adjacent to each plot and not nearer than 5 feet to plants to be inoculated was set a group of three controls, one of which was of the species from which the inoculum to be used was to be isolated. Suitable cultivation was provided throughout the season, and the plants grew vigorously.

APPARATUS.—In order to insure favorable humidities and temperatures for infection, it was necessary to devise special apparatus. Accordingly, two movable moist chambers, 6 to 8 feet long, 3½ feet wide, and 3½ to 4 feet high, were constructed so that they could be set over the experimental plants. One was made of glass and wood (see fig. 2), and the other of light galvanized iron over a wooden frame. The latter was provided with glass doors which admitted enough light to keep the plants under fairly normal conditions while covered. These chambers were

[1] In this paper the experimental plants are listed in all cases in the order most advantageous for comparing results from the inoculation tests. When two or more varieties or strains of the same species were used, they were designated, respectively, by appropriate letters (A, B, etc.). In nomenclature Wight's usage was followed where applicable. (WIGHT, W. F. NATIVE AMERICAN SPECIES OF PRUNUS. U. S. Dept. Agr. Bul. 179, 75 p., 4 fig., 13 pl. 1915) The writer wishes to express his indebtedness to Mr. Wight for personal advice in these matters and for verifying the identification of certain species. In varietal names of plums and cherries, the usage of Hedrick was followed. (HEDRICK, U. P. THE PLUMS OF NEW YORK. 616 p., col. pl. Albany, N. Y., 1911. AND HEDRICK, U. P. THE CHERRIES OF NEW YORK. 371 p., col. pl. Albany, N. Y., 1915.)

draped with cheesecloth, which throughout all clear days of operation was kept continuously moist by small streamlets of water from pipes which were conveniently attached to the ridges of the slanting roofs. The evaporation from the cheesecloth, even on the hottest days, kept the temperatures within the chambers below 32° C., while the excess of water kept the soil under them saturated. During the day the temperatures ordinarily ranged between 20° and 28° C., and the relative humidities between 90 and 100 per cent. At night the temperatures were generally lower, while the humidities usually reached or closely approximated saturation. While these chambers gave good results, their weight proved to be a serious practical disadvantage, and in 1917 they were replaced by the much more convenient apparatus described on page 546.

The inocula were applied by means of atomizers, the construction of which was satisfactory and particularly well adapted to steam sterilization.

INOCULA.—When the inocula were procured from cultures, spore suspensions were prepared by introducing sterile distilled water into the culture tubes and teasing the spores into suspension. Such suspensions, suitably diluted with sterile distilled water, comprised the inocula. All cultures used were from single-spore strains. For direct inoculations, spore suspensions were prepared by removing the contents of acervuli by means of sterile scalpels to sterile distilled water, in which they were stirred and suitably diluted.

Just before the inoculations were made, drops of the suspensions to be used were placed on clean sterile glass slides in moist chambers and tested for viability of spores.

METHOD OF INOCULATION.—The inoculations were always made late in the afternoon. The moist chambers already described were placed over the experimental plants and the ground inside was abundantly watered. The inoculum was then applied as spray by means of the sterile atomizers. The chambers were promptly closed and were kept continually wet on the outside by means of the devices already described. Unless otherwise stated, they were always left in place for two days.

Of the controls, series 1 was treated in every way like the inoculated plots, except that sterile distilled water was used instead of an inoculum. The additional special controls, which were located near each plot in the manner already described (p. 540), were left untreated.

RESULTS OF EXPERIMENTS OF 1916

A summary of the experiments of 1916 appears in Table I.

Of the 30 control plants, which included all the experimental species and varieties except *P. padus*, which died, only one, *P. avium*, had developed any infection when the final notes were made on October 14.

Between September 11 and October 14, infection appeared on several leaves of this plant. The lateness of its occurrence and its scarcity, however, make this slight chance infection negligible in the interpretation of the season's results. This conclusion is further justified by the fact that the scores of inoculated trees which did not develop infection may be considered as supplementary controls.

The results of these inoculations, as is witnessed by the abundant infection of the more susceptible plants in series 4, 5, 6, and 7, show that the conditions of the experiments were favorable for infection. There was no indication, however, that these conditions were markedly abnormal or that they should be expected to predispose the plants to infection.

The infection in 1916 was uniformly distinctly less severe than in 1917 (Tables I–VIII). While the reasons for this fact are not yet fully understood, there is considerable evidence to indicate that one or both of the following factors may have been concerned: (1) The probable diminution in pathogenicity of the parasites in question after a considerable period in culture, and (2) a less vigorous germination of their spores in concentrated than in dilute suspensions.

There is considerable evidence (Tables I–VIII) that a relatively long period in culture tends to lessen the pathogenicity of the fungi under investigation, although in vigorously sporulating fresh cultures they are highly pathogenic. This evidence, however, is neither sufficiently extensive nor consistent to warrant final conclusions, and it would appear that other factors than the mere age of a strain in culture are concerned. Cultures of the same age and from the same source may, for instance, vary greatly in the vigor of their sporulation, and consequently in their effectiveness as sources of inocula.

Experiments, the details of which are reserved for a later paper, have shown conclusively that in laboratory tests spores may fail to germinate in a moderately dilute suspension, while in a more dilute portion of the same suspension under like conditions vigorous germination may occur. This condition was not fully apprehended when the experiments of 1916 and the earlier greenhouse series of 1917 were conducted, though the germination experiments in connection with these series show that the suspensions used could not have been excessively concentrated. In all subesquent experiments great care was taken to guard against too great concentration of the inocula. It is hoped that further attention may be given to these problems.

The detailed results of the inoculations are best apprehended by a perusal of the tables. To facilitate this end, the same form and headings and, so far as feasible, the same footnotes have been used in Tables I to VIII.

TABLE I.—*Summary of inoculation experiments with Coccomyces spp., Madison, Wis., 1916*

INOCULATIONS [a]

Series.	Date.	Inoculum.					
		Original host.	Spores.	Source.	Age of strain in culture.	Germination after 2 days.[b]	
						Per cent.	Vigor.
					Months.		
1	July 14			Not inoculated			
2	July 3	*P. domestica*	Conidia	14-day-old culture	10.5	10	+
3	Aug. 28	*P. serotina*	do	*P. serotina*, leaves, Madison.		50	+
4	July 10	*P. mahaleb*	do	14-day-old culture	10.9	10	+
5	July 18	*P. virginiana*	do	*P. virginiana*, leaves, Sturgeon Bay.		3	+
6	July 20	*P. cerasus*	do	14-day-old culture	14.2	95	++
7	Aug. 18	*P. avium*	do	13-day-old culture	3.1	95	+

RESULTS

Plant and treatment.	Infection.[c, g]						
	Series 1 (not inoculated).	Series 2 (inoculum from *P. domestica*).	Series 3 (inoculum from *P. serotina*).	Series 4 (inoculum from *P. mahaleb*).	Series 5 (inoculum from *P. virginiana*).	Series 6 (inoculum from *P. cerasus*).	Series 7 (inoculum from *P. avium*).
Inoculated:							
P. cerasus		0	0	4	0	*f* 1	*i* 2
P. avium A		0		5	0		
P. avium B		0	0	4	0	*f* 5	*i* 4
P. mahaleb			2	2	3	*f* 1	*i* 2
P. pennsylvanica		0	0	*d* 0	0	*e* 0	
P. serotina		0		0	0	0	
P. padus		0					
P. virginiana		0	0		4	0	0
P. domestica		*i* 2	0	0	0	0	
P. insititia		*i* 2	0	0	*f* 2	0	0
P. americana A		0	0	0	0	0	0
P. americana B		0	0	0	0	0	0
P. salicina		0	0	0	0	0	0
P. munsoniana		0	0	0	0	*f* 4	*e* 0
A. persica		0	0	0	0	0	0
A. persica nectarina		0	0	0	0	0	0
P. armeniaca		0	0	0	0	0	0
P. besseyi		0	0	0	0	*f* 4	*f* 4
Not inoculated:							
P. cerasus	0						
P. avium B	*h* (?)	0		0			0
P. mahaleb	0	0		0		0	
P. pennsylvanica	0						
P. serotina	0						
P. virginiana	0				0		
P. domestica	0	0			0	0	0
P. insititia	0						
P. americana A	0						
P. americana B	0		0	0			
P. salicina	0						
P. munsoniana	0						
P. cerasifera	0						
A. persica	0						
A. persica nectarina	0						
P. armeniaca	0						

[a] For details of technic, see p. 540.

[b] Approximate vigor is arbitrarily represented by symbols, as follows: −=weak; +=vigorous; ++=very vigorous. Percentage of germination estimated.

[c] Severity of infection is indicated by numbers, as follows: 1=scattered, usually 1 or 2 lesions per leaf; 2=general, averaging 2 to 5 per square inch of leaf surface; 3=general, averaging 6 to 15 per square inch of leaf surface; 4=general, averaging 16 to 50 (estimated) per square inch of leaf surface; and 5=general, averaging more than 50 (estimated) per square inch of leaf surface. In cases where the infection on a given plant was not uniform, only the more severely affected leaves were considered (Pl. 55). The negative results of series in which the maximum infection is represented by a number less than 3 are printed in **bold-face** type, and are not included in the summary (Table IX). All other results, unless otherwise noted, are printed in italic type and are included in the summary.

[d] After a prolonged incubation period small brownish flecks appeared (see p. 544).

[e] After a prolonged incubation period spots developed, but the fungus failed to fructify (see p. 544).

[f] Incubation period prolonged.

[g] The final notes were made on October 14.

[h] See discussion on p. 542.

[i] Secondary infection was abundant.

The fungus from *P. domestica* (series 2) induced infection only on *P. domestica* and *P. insititia*. The low degree of infection on the original host, however, makes it unsafe to place confidence in the negative results of this series (compare Table VI and see footnote c, Table I). It is probable that diminished pathogenicity of the fungus in culture, in connection with other factors not yet fully understood, is responsible for the relative sparseness of this infection.

In series 3 the fungus from *P. serotina* infected only *P. mahaleb*. Had *P. serotina* of this bed lived, it would undoubtedly have been abundantly infected (compare Table VIII).

The fungus from *P. mahaleb* (series 4) induced abundant infection on *P. avium* and *P. cerasus* and relatively sparse infection on *P. mahaleb*. After a prolonged incubation period, small flecks appeared on the inoculated leaves of *P. pennsylvanica*, but no spots developed, and the fungus did not fructify. The slow development upon inoculated leaves of flecks or small spots upon which there was no fungal fructification was of fairly common occurrence in these experiments. While satisfactory histological studies of such lesions have not yet been completed, the available evidence indicates that such cases (adequately controlled) may be considered as aberrant infection. Such infection appears to occur commonly only in the case of cross inoculations which rarely, if ever, result in typical infection. Flecking and spotting of this type, furthermore, were much more common in the greenhouse inoculations late in the summer when the plants were weakening than in outdoor experiments. The gradation of this apparently aberrant infection into normal infection made it necessary to adopt a criterion of infection. Accordingly fructification of the fungus was adopted as the most conveniently workable criterion which suggested itself. Unless the fungus fructified, therefore, results are recorded as negative, with annotations regarding flecking or spotting.

In series 4 the infection was more severe than in series 2, despite the fact that the inoculum was from a strain which had been slightly longer in culture than that used in series 2. This is in accord with the belief already expressed that other factors than the age of a strain in culture may influence the diminution of its pathogenicity. In this case, for instance, the strain from *P. mahaleb* had maintained its ability to sporulate in culture in a much higher degree than had that from *P. domestica*. To avoid such complications, the later inoculations were made, so far as feasible, with recently isolated, vigorously sporulating strains. It should be noted, however, that the slow growth of these fungi in culture offers a serious handicap in this regard, as a period of one to two months is ordinarily necessary for isolating a single-spore strain and growing it in sufficient quantity to furnish the necessary inoculum.

In series 5 the fungus from *P. virginiana* induced abundant infection on its original host, moderate infection on *P. mahaleb*, and light, delayed infection on *P. insititia*.

In series 6, inoculated with a 14-month-old strain from *P. cerasus*, infection was rather sparse and somewhat slow in developing, despite the fact that the inoculum contained abundant, vigorously viable spores. Abundant infection developed on *P. avium* and sparse infection on *P. cerasus* and *P. mahaleb*. Rather abundant infection developed after a more prolonged incubation period on *P. munsoniana* and *P. besseyi*.

The fungus from *P. avium* (series 7) infected *P. avium* abundantly and *P. cerasus* and *P. mahaleb* rather sparsely. After a prolonged incubation period, *P. besseyi* also developed abundant infection.

Numerous reisolations were made in the same manner as in 1917 (p. 563), and with like results.

EXPERIMENTS OF 1917

In 1917 the outdoor tests were supplemented by extensive greenhouse experiments. By starting potted plants early in the spring, it was found possible to have them in leaf as soon as the ascospores of the fungi under investigation approached maturity (their maturity can be hastened in the laboratory). Thus, it was possible to conduct extensive experiments, without danger of natural infection, before the outdoor plants were ready. In this way essentially the equivalent of two years' data was secured in one season. Furthermore, it was possible to study infection under varied experimental conditions more readily in the greenhouse than out of doors. The danger of falling into errors of interpretation of the results obtained under greenhouse conditions seems adequately guarded against by the extensive outdoor tests of two seasons.

TECHNIC OF INOCULATIONS

OUTDOOR EXPERIMENTS

EXPERIMENTAL PLANTS.—The following plants were used: *Prunus cerasus* (Montmorency Stark), *P. avium* (Yellow Spanish), *P. mahaleb*, *P. pennsylvanica*, *P. serotina* A, *P. serotina* B, *P. serotina* C, *P. padus*, *P. virginiana*, *P. domestica* (Lombard), *P. insititia* (Damson Shropshire), *P. americana* A (De Soto), *P. americana* B, *P. salicina* (Burbank), *P. munsoniana* (Wild Goose), *Amygdalus persica* (Elberta), *P. armeniaca* (Superba), and *P. besseyi*.

Two consignments of *P. serotina* which were received under erroneous labels were included in the experiments for purposes of comparison. Plants of *P. serotina* were accordingly distinguished as to source by the letters "A," "B," and "C."

Except in cases where varietal names are given, the experimental plants were seedlings or cuttings. They were obtained, assembled, and

heeled in in accordance with the method adopted in the preceding year's work (p. 540). *P. pennsylvanica* and *P. virginiana* were collected in the vicinity of Madison. In late May, after being severely pruned, the plants were set in a convenient field, well removed from any known source of natural inoculum of the *Coccomyces* spp. under investigation. The plants to be inoculated were set in groups, each of which contained all of the experimental species and varieties, conveniently disposed for covering with a moist chamber. They formed in each plot two rows, 14 inches apart, with 10-inch intervals between plants in the rows. A similarly arranged group of control plants was placed 6 feet from one end of each of these plots. The number and identity of the controls varied with the inocula to be used. It was planned to have in each control group a cultivated cherry, a wild cherry, and a plum, including the host from which the inoculum to be used was to be secured. Further controls were added as the available supply of plants permitted. Inasmuch as cross-inoculation tests with *Cladosporium* spp. on certain species of Prunus were being conducted at the same time, the plants to be inoculated with *Cladosporium* spp. were set with the controls of the Coccomyces inoculations. Thus, they served as additional controls for the latter series, the inoculated plants of which, in turn, served as controls for the Cladosporium series. All beds were at least 6 feet apart. Suitable cultivation was provided throughout the season, and the plants which took root grew vigorously. However, owing to the bad condition in which some of the nursery stock arrived, many plants failed to take root. This marred considerably the completeness of the experiments.

Apparatus.—The moist chamber illustrated in figure 1 was used to cover the inoculated plants, while a smaller chamber of similar construction covered the controls. This latter chamber was 3½ feet wide, 6 feet long, and 4½ feet high. Its roof, however, was pyramidal, rising to a peak upon which the play of a single nozzle provided an adequate supply of water. These chambers were connected by a 100-foot hose to faucets conveniently located on a temporary pipe line. They were easily handled by two men, and were very satisfactory.

In series 1, A–D, in which the plants were inoculated in their natural habitat, a bell jar lined with moist paper towels was placed over each plant.

The inocula were applied by means of sterile atomizers equipped with 8-ounce containers and long intake tubes. In some of the experiments the atomizers were operated by compressed air from a small portable drum, the pressure being regulated by a cut-off. The atomizers were connected with the drum by a 10-foot length of silk-covered rubber tubing. This apparatus gave very good results until a defect in the drum led to its disuse. A light drum with a capacity of 6 to 10 cubic feet, constructed to withstand a pressure of at least 30 pounds, and con-

veniently mounted on a small truck which will easily pass through standard doorways, will probably be much more satisfactory. A supply of compressed air with which to charge such apparatus is highly desirable, since charging it by means of an automobile pump is as laborious and time-consuming as atomizing by hand.

INOCULA.—The inocula were prepared in the same fashion as in the experiments of 1916 (p. 541), except that in a few cases the cultures used were not from single-spore strains. In all cases, however, the isolations were made by the method previously described by the writer,[1] their purity being established by frequent microscopic examinations in their

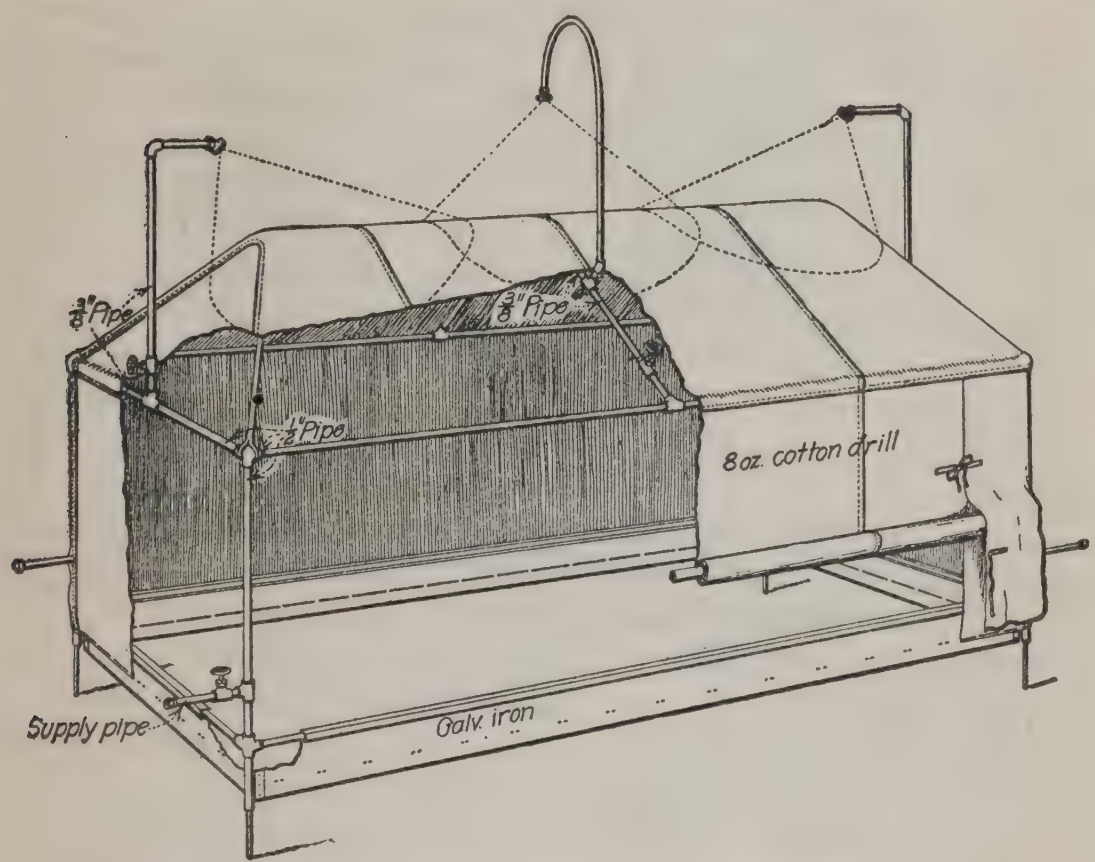


FIG. 1.—Moist chamber used in the outdoor inoculation experiments of 1917.

early stages of development and by later cultural tests. Inasmuch as no differences were observed between the results from single- and multiple-spore cultures, the strains are not distinguished in this regard in the tables.

METHOD OF INOCULATION.—The plants were inoculated by the method used in 1916 (p. 541). The moist compartments, however, were left in place only one day.

Before each inoculation, in order to facilitate the taking of records, markers of white string were tied near the apexes of several twigs on each plant. In this way the results of successive inoculations on the same

[1] KEITT, G. W. SIMPLE TECHNIQUE FOR ISOLATING SINGLE-SPORE STRAINS OF CERTAIN TYPES OF FUNGI. *In* Phytopathology, v. 5, no. 5, p. 266–269, 1 fig. 1915.

plants were usually readily differentiated, such inoculations being made on the recently developed foliage of the rapidly growing twigs.

GREENHOUSE EXPERIMENTS

EXPERIMENTAL PLANTS.—The experimental plants were from the same sources and of the same species and varieties as those used in the outdoor tests (p. 545). They did not, however, include all the species used in the field. The plants were assembled in early April, severely

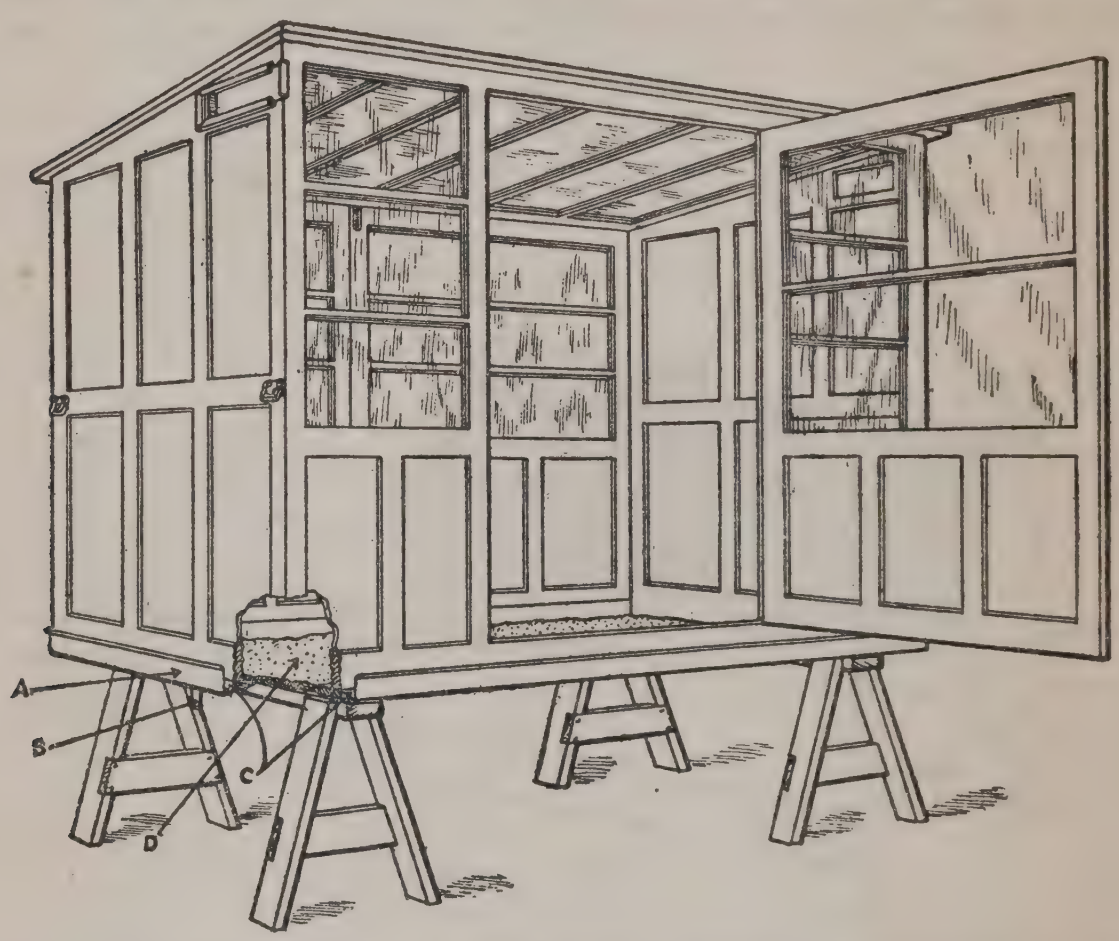


FIG. 2.—Moist chamber used in the greenhouse inoculation experiments: *A*, galvanized-iron pan, with drainage outlet *B*; *C*, strips of board to support chamber; *D*, sphagnum or sand. Dimensions: Length, 6 feet; breadth, 32 inches; height of front, 4½ feet. This chamber was also used in the outdoor inoculations of 1916 (p. 540).

pruned, and set in 6- to 10-inch flowerpots. The pots were submerged in the soil in a greenhouse of the garden type, and the plants were given suitable cultural attention. To inhibit the development of insect pests, particularly the red spider, the plants were washed daily with a strong spray from an angle nozzle conveniently connected with the water supply. Throughout the early spring the temperature of this greenhouse ordinarily ranged between 21° and 27° C. In late spring and early summer the maximum temperature was frequently considerably higher.

APPARATUS.—Suitable conditions of humidity were obtained by placing the inoculated plants in the moist chamber illustrated in figure 2.

While, in order to avoid excessively high temperatures, it was necessary to screen this chamber against direct sunlight, the experimental plants received a good supply of diffuse light. This apparatus gave very satisfactory results, maintaining a humidity which closely approximated saturation.

All spore suspensions were applied by atomizers, as in the outdoor series (p. 547).

In making inoculations with ascospores, application of the spores by means of natural discharge of the asci was by far the most satisfactory method tried. This was accomplished by adaptation of methods which have been used by others.[1] The simple device used is illustrated in figure 3. The washers were cut from gasket rubber by means of cork borers, which were also used for cutting the ascogenous leaf fragments. The cover glasses were standard No. 2 squares (22 mm.). The clips were made from No. 24 brass wire, and furnished a grip which, while adequate for holding the apparatus in place, was not strong enough to injure the leaves. These devices gave very satisfactory results (Pl. 59, C, D).

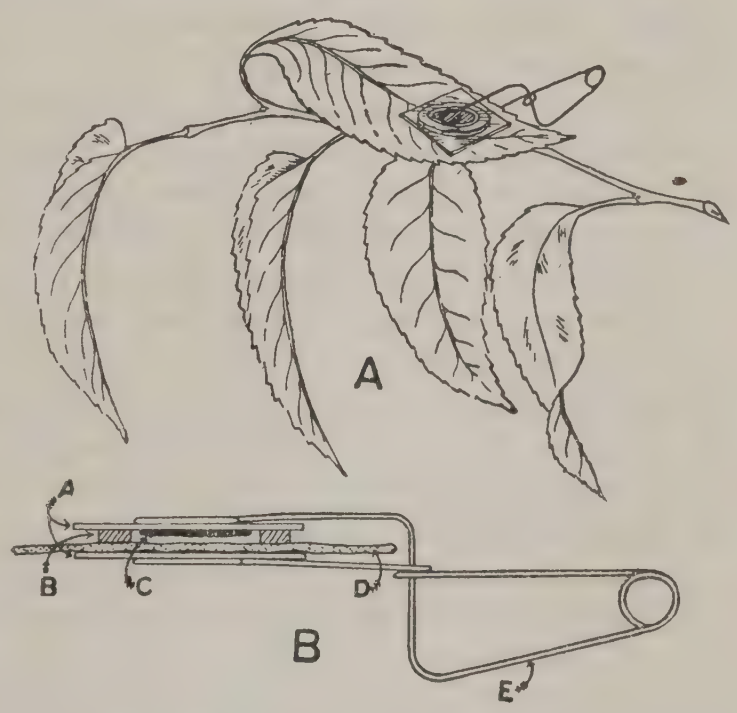


FIG. 3.—Device used for inoculation by means of natural ejection of ascospores: *A*, on *P. americana*; *B*, in cross section (see text). *a*, cover glass; *b*, rubber washer; *c*, ascogenous leaf fragment; *d*, leaf to be inoculated; *e*, clip.

INOCULA.—Unless otherwise noted, all inocula were prepared like those of the outdoor experiments (p. 546).

METHOD OF INOCULATION.—In all series inoculated with spore suspensions the experimental plants were removed to the pathologium,[2] where they were sprayed in the same manner as the outdoor series (p. 547) and placed in the moist chamber. Similarly, in the inoculations by discharge of asci, the experiments were set up in the pathologium, and the plants were then promptly placed in the moist compartment. The inoculations were made on the lower (dorsal) surfaces of the leaves, which were left in such position that the spores might be discharged downward upon them rather than upward. This was easily accomplished after the plants had

[1] Particularly helpful suggestions in this regard were kindly given by Dr. F. R. Jones, now Pathologist, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture, and by Prof. James Johnson, of the University of Wisconsin.

[2] Pathologium: A phytopathological laboratory used in conjunction with a greenhouse.

been placed in the moist chamber by propping the leaves against adjacent leaves or twigs (fig. 3). Ordinarily several cells were placed upon each plant, and in order to increase the number of trials, they were moved at suitable times to other leaves. By shifting the cells from one plant to another within the series when these changes were made, the chances of failure of discharge of spores upon any given plant were minimized. Just before each experiment was begun, each leaf fragment of the ascogenous material to be used was tested for spore discharge, and unless spores were being discharged abundantly the material was rejected. Even with these precautions, however, it was not possible to insure a satisfactory inoculum in every case. Therefore, full confidence can not be placed in each individual negative result. Great care was exercised to maintain within the cells favorable conditions of humidity for discharge of the asci. The leaf fragments, bearing fresh, open ascocarps, were always taken directly from the moist chambers. They were wet just enough to make them adhere well to the cover glasses. A white string about the petiole of each inoculated leaf served as a marker, and in each series all the inoculations were made on approximately the same part of the leaf surface, usually, for convenience, near the apex. Freshly discharged ascospores were found to be so uniformly viable that in most cases special germination tests were not made. The inoculated plants were held in the moist chambers for two days, after which they were incubated in the greenhouses. No inoculated plant or other source of possible inoculum was carried into the greenhouse where the uninoculated plants were grown, and the various inoculated series were kept well apart in the other greenhouses, in order to minimize the possibilities of chance infection. In these greenhouses throughout the early spring the temperatures ordinarily ranged between 24° and 29° C. In late spring and early summer the maximum temperatures were considerably higher, but not excessive. In late July and August, despite careful attention to shading and ventilation, the temperatures at times became excessive. The more important aspects of the experiments, however, were completed before this time.

The control plants were sprayed with sterile distilled water, and held in the moist chamber for two days.

RESULTS OF EXPERIMENTS OF 1917

The results of the experiments of 1917 are summarized in Tables II to VIII.

Of the scores of control plants of both the outdoor and the greenhouse series, including representatives of all the species and varieties used, not one developed a single infection throughout the entire season. Futhermore, the many inoculated plants upon which no infection occurred serve as additional controls. It is evident, therefore, that the experiments were adequately and satisfactorily guarded against chance infec-

tion. The remoteness of the experimental plants from infected species of Prunus apparently precluded chance natural infection by ascospores, while the delicate nature of the conidia of these fungi, and their methods of dissemination and infection, account in large measure for the lack of spread of the disease from plants of one series to those of another.

The abundant infection which occurred on susceptible plants both in the outdoor and the greenhouse series proves conclusively that the conditions of the experiments were favorable for infection. There was, however, no evidence to indicate that these conditions were such as to predispose the experimental plants to infection, with the possible exception of some cases of infection in the greenhouse after much prolonged incubation periods and after the experimental plants had become somewhat lowered in vitality. Under such conditions rare or difficult crosses occurred more freely in the greenhouse than outside, and in several such cases crosses which have not been duplicated outdoors were effected in the greenhouse. Such results are interpreted in the light of these facts and are suitably annotated in the tables. With these exceptions, the results of the greenhouse series closely parallel those of the outdoor experiments, and appear to be fully reliable.

The results of the year's inoculations are best apprenended by a study of Tables II to VIII. Each table contains a summary of all the inoculations with strains obtained from one host. By reading down the columns of the lower section, one may quickly trace the results by series, while, by reading across columns, he may compare the results of all the series on any species. The details regarding inoculations may be ascertained by reference to the upper section of the table.

TABLE II.—*Summary of inoculation experiments with Coccomyces spp. from Prunus cerasus, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination.[b]		
						After hours.	Per cent.	Vigor.
				Months.				
Out of doors:								
1A	June 8	Ascospores.	Leaves, Madison	.....	Dehiscence.	.....	.....	.....
10	Aug. 17	Conidia	11-day-old culture	1.7	Spray	24	5	+
15	Sept. 19	do	10-day-old culture [i]	1.8	do	24	1	+
In greenhouse:								
101	May 9	do	20-day-old culture	7.6	do	20	60	+
101	June 2	Ascospores.	Leaves, Madison	.....	Dehiscence.	.....	.....	.....
101	July 2	Conidia	20-day-old culture	1.0	Spray	17	50	++
104	May 16	do	14-day-old culture	7.8	do	20	90	++
104	June 5	Ascospores.	Leaves, Madison	.....	Dehiscence.	.....	.....	.....
104	June 26	Conidia	22-day-old culture	.7	Spray	24	1	+
104	July 15	do	15-day-old culture	1.3	do	24	1	+
106	May 19	Ascospores.	Leaves, Madison	.....	Spray [k]	48	Trace	−
106	June 9	do	do	.....	Dehiscence.	.....	.....	.....

[a] For details of technic, see p. 545.
[b] See footnote b, Table I.
[i] This strain was reisolated from *P. cerasifera* of series 104.
[k] Crushed ascocarps in sterile distilled water.

TABLE II.—*Summary of inoculation experiments with Coccomyces spp. from Prunus cerasus, Madison, Wis., 1917*—Continued

RESULTS

Plant and treatment.	Infection. [c, g]											
	Out of doors.			In greenhouse.								
	Series 1A. [h]	Series 10.	Series 15.	Series 101 from inoculation of—			Series 104 from inoculation of—				Series 106 from inoculation of—	
				May 9.	June 2.	July 2.	May 16.	June 5.	June 26.	July 15.	May 19.	June 9.
Inoculated:												
P. cerasus	.....	*5*	*4*	**0**	*5*	*5*	**0**	*4*	*4*	*5*	**0**	*4*
P. mahaleb	.....	.....	*4*	**0**	*0*	*3*	*1*	*3*	*4*	*4*	*1*	*3*
P. pennsylvanica	*d 0*	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. serotina A	.....	.....	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. serotina B	.....	*0*	*0*	**0**	*0*	*0*	**0**	*0*	*0*	*0*	**0**	*0*
P. serotina C	.....	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. padus	.....	*0*	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. virginiana	*0*	*0*	.....	**0**	*0*	*0*	**0**	*0*	*0*	*0*	**0**	*0*
P. domestica	.....	*0*	*0*	**0**	*e 0*	*e 0*	**0**	**0**	*e 0*	*e 0*	**0**	*e 0*
P. insititia	.....	*0*	*0*	**0**	*j* (?)	*f 3*	**0**	(?)	*f 4*	*f 4*	**0**	*f 3*
P. americana A	.....	*0*	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. americana B	.....	*0*	*0*	**0**	*d 0*	*d 0*	**0**	**0**	*d 0*	*d 0*	**0**	*d 0*
P. salicina	.....	*0*	*0*	**0**	*e 0*	*e 0*	**0**	*0*	*0*	*0*	**0**	*e 0*
P. munsoniana	.....	.....	*0*	**0**	*f 3*	*f 3*	**0**	(?)	(?)	*f 4*	**0**	*e 0*
A. persica	.....	*0*	*0*	**0**	*0*	*0*	.....	.....	.....	.....	**0**	*0*
P. besseyi	.....	*0*	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. mahaleb (*P. cerasus* stock)	.....	.....	.....	.....	.....	.....	**0**	*2*	*3*	*5*	.....	.....
P. cerasifera (*P. domestica* stock)	.....	.....	.....	.....	.....	.....	**0**	(?)	(?)	*f 4*	.....	.....
Not inoculated:												
P. cerasus	.....	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
P. americana B	.....	*0*	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. domestica	.....	*0*	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. mahaleb	.....	.....	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. pennsylvanica	*0*	.....	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. serotina	.....	.....	*0*	.....	.....	.....	.....	.....	.....	.....	*0*	*0*
A. persica	.....	*0*	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. virginiana	*0*	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

c–f See footnotes c to f, Table I.

g The final notes on inoculations of 1917 were made on the following dates: Series 1 A–D, June 26; all other outdoor series, October 15; all greenhouse series, August 18.

h See discussion, p. 551.

j In cases where it was not possible to differentiate the results of successive inoculations on the same plant, the combined results of all the inoculations in question are printed in italic type, under the heading of the last inoculation in question, with the appropriate footnote, d, e, or f, and the earlier indistinguishable results are recorded in **bold-face** type.

DISCUSSION OF TABLE II

Abundant infection resulted from all inoculations except the following: Series 1A; series 101, May 9; series 104, May 16; and series 106, May 19. The absence of infection in series 1A was evidently due to the fact that this series contained no susceptible plant. Lack of virulence of the inocula probably accounts for the failures in series 101 and 104, the strain used having been more than seven months in culture. In series 106 the inoculum was evidently unsatisfactory, only a trace of germination occurring in the laboratory test. In these and like series, therefore, except series 1A, the negative results are without value and are excluded from the summary (Table IX), while the positive results are not comparable in regard to the severity of infection with those obtained

from inoculations made under more favorable conditions. Consequently such positive results are left out of account in computing the average degrees of infection (Table IX. See footnote c, Table I).

P. cerasus was uniformly abundantly infected (Pl. 59, A), while on *P. mahaleb* (Pl. 56, C) the infection was only slightly less severe. In the greenhouse, after prolonged incubation periods, *P. insititia* (Pl. 56, B) consistently developed infection, but this cross did not occur in the field trials. With *P. munsoniana* (Pl. 56, D) similar results were obtained. In 1916, after prolonged incubation (Table I), this cross led to infection in the field. The single greenhouse test with *P. cerasifera* (Pl. 56, A) led to delayed infection. On the other inoculated plants and on the controls (Pl. 59, B), no infection developed, though in the greenhouse after prolonged incubation periods small spots developed in considerable abundance upon *P. domestica* (Pl. 56, E) and *P. salicina* and flecks upon *P. americana* B. Similarly, flecks developed upon *P. pennsylvanica* out of doors (see p. 544). In none of these cases, however, did fructification of the fungus accompany these developments.

DISCUSSION OF TABLE III

No infection resulted from the first inoculation of series 102, and only slight infection on *P. cerasus* was induced by the first inoculation of series 14. From all the other inoculations moderate infection developed, but in no case was it severe. The causes of these variations are not fully understood. It seems probable that this generally low degree of infection was due, in considerable part at least, to a diminution in the pathogenicity of the fungus in culture, since a single strain, more than a year old, was used for all these experiments. Such reduction in pathogenicity, however, would not explain the differences in the results of the first and the subsequent inoculations of series 102. It is possible that temperature variations in the greenhouses during the course of these experiments may have been an important factor, as temperature differences might well cause more conspicuous variations in the case of a strain of reduced pathogenicity than with a virulent strain. It should also be remembered that, owing to the failure of *P. avium* stock to root, the original host was not included in these tests. These matters, however, are subjects for further investigation.

Infection occurred consistently on *P. cerasus* and *P. mahaleb*. None of the other experimental plants were infected, though in certain cases, after prolonged incubation periods, spots developed upon *P. padus*, *P. domestica*, *P. insititia*, and *P. munsoniana*, and flecks appeared on *P. americana* A and B and *P. salicina*.

TABLE III.—*Summary of inoculation experiments with Coccomyces spp. from Prunus avium, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination.[b]		
						After hours.	Per cent.	Vigor.
				Months.				
Out of doors:								
11	July 30	Conidia	9-day-old culture	14.5	Spray	24	75	+
11	Aug. 29	...do	14-day-old culture	15.5	...do	.....	.....	.....
14	Aug. 3	...do	11-day-old culture	14.6	...do	17	90	+
14	Sept. 12	...do	12-day-old culture	15.9	...do	24	95	+
In greenhouse:								
102	May 12	...do	9-day-old culture	11.9	...do	20	50	+
102	May 24	...do	15-day-old culture	12.3	...do	.....	.....	.....
102	July 2	...do	18-day-old culture	13.6	...do	17	75	+

RESULTS

Plant and treatment.	Infection.[c, g]						
	Out of doors.				In greenhouse.		
	Series 11 from inoculation of—		Series 14 from inoculation of—		Series 102 from inoculation of—		
	July 30.	Aug. 29.	Aug. 3.	Sept. 12.	May 12.	May 24.	July 2.
Inoculated:							
P. cerasus	3	3	[h] 2	[h] 3	0	4	4
P. mahaleb	1	3	0	3	0	3	3
P. pennsylvanica	0	0	0	0	.....	.....	.....
P. serotina A	0	0	0	0	.....	.....	.....
P. serotina B	0	0	0	0	0	0	0
P. serotina C	.....	.....	0	0	.....	.....	.....
P. padus	[e] 0	0	0	0	.....	.....	.....
P. virginiana	0	0	.....	.....	0	0	0
P. domestica	0	0	0	0	0	0	[e] 0
P. insititia	0	0	0	0	0	0	[e] 0
P. americana A	[d] 0	0	0	0	.....	.....	.....
P. americana B	[d] 0	0	0	0	0	0	[d] 0
P. salicina	0	0	0	0	0	0	[d] 0
P. munsoniana	0	0	0	0	0	0	[e] 0
A. persica	0	0	0	0	0	0	0
P. armeniaca	0	0	.....	.....	.....	.....	.....
P. besseyi	0	0	0	0	.....	.....	.....
Not inoculated:							
P. cerasus	0	0	0	0	0	0	0
P. domestica	0	0	0	0	.....	.....	.....
A. persica	0	0	0	0	.....	.....	.....
P. americana B	0	0	0	0	.....	.....	.....
P. virginiana	0	0	.....	.....	.....	.....	.....
P. salicina	.....	.....	0	0	.....	.....	.....
P. serotina	.....	.....	0	0	.....	.....	.....
P. padus	0	0	.....	.....	.....	.....	.....
P. avium	0	0	.....	.....	.....	.....	.....
P. armeniaca	0	0	.....	.....	.....	.....	.....

[a] See footnote a, Table II.
[c] See footnotes b to e, Table I.
[g] See footnote g, Table II.
[h] Secondary infection was very abundant.

TABLE IV.—*Summary of inoculation experiments with Coccomyces spp. from Prunus mahaleb, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination.[b]		
						After hours.	Per cent.	Vigor.
Out of doors:				*Months.*				
7	July 25	Conidia	*P. cerasus* leaves, series 116.	.....	Spray	24	30	+
7	Aug. 16	...do	11-day-old culture	2.4	...do	24	−1	−
8	July 26	...do	*P. mahaleb* leaves, series 116.	.....	...do	20	50	+
8	Aug. 8	...do	12-day-old culture	2.1	...do	48	25	+
18	Sept. 22	...do	15-day-old culture	3.5	...do	17	0	.....
In greenhouse:								
115	June 30	...do	24-day-old culture	.8	...do	15	50	+
115	July 10	...do	10-day-old culture	1.1	...do	48	5	+
115	July 26	...do	*P. mahaleb* leaves, series 116.	.....	...do	20	50	+
116	July 4	...do	20-day-old culture	1.0	...do	.....	.....	.....
116	July 12	...do	11-day-old culture	1.3	...do	48	−1	−

RESULTS

Plant and treatment.	Infection. [c, g]									
	Out of doors.					In greenhouse.				
	Series 7 from inoculation of—		Series 8 from inoculation of—		Series 18.	Series 115 from inoculation of—			Series 116 from inoculation of—	
	July 25.	August 16.	July 26.	August 8.		June 30.	July 10.	July 26.	July 4.	July 12.
Inoculated:										
P. cerasus	4	4	4	4	4	3	5	3	4	4
P. avium	.....	.....	5	5	.....	.....	.....	.....	.....	.....
P. mahaleb	h 3	h 3	h 2	h 2	3	2	2	4	3	3
P. pennsylvanica	2	1	f 3	4	.....	.....	.....	.....	.....	.....
P. serotina A	0	0	0	0	.....	.....	.....	.....	.....	.....
P. serotina B	.....	.....	0	0	.....	0	0	0	0	0
P. serotina C	0	0	0	0	.....	.....	.....	.....	.....	.....
P. padus	e 0	e 0	e 0	e 0	0	.....	.....	.....	.....	.....
P. virginiana	0	.....	0	0	.....	0	0	0	0	0
P. domestica	0	0	0	0	0	0	e 0	0	e 0	e 0
P. insititia	0	0	0	0	0	(?)	f 2	0	(?)	f 2
P. americana A	0	0	0	0	0	.....	.....	.....	.....	.....
P. americana B	0	0	0	0	0	0	0	0	0	d 0
P. salicina	0	0	.....	.....	0	.....	.....	.....	.....	.....
P. munsoniana	0	e 0	0	0	0	f 2	f 3	0	(?)	f 3
A. persica	e 0	e 0	0	0	0	0	0	0	0	0
P. armeniaca	0	0	0	0	.....	.....	.....	.....	.....	.....
P. besseyi	0	0	3	0	0	.....	.....	.....	.....	.....
P. mahaleb (*P. cerasus* stock)	.....	.....	.....	.....	.....	.....	.....	.....	3	3
Not inoculated:										
P. mahaleb	0	0	0	0	0	0	0	0	0	0
P. cerasus	0	0	.....	.....	0	.....	.....	.....	.....	.....
P. americana B	0	0	0	0	.....	.....	.....	.....	.....	.....
P. domestica	0	0	0	0	.....	.....	.....	.....	.....	.....
P. virginiana	0	0	0	0	.....	.....	.....	.....	.....	.....
P. armeniaca	0	0	.....	.....	.....	.....	.....	.....	.....	.....
A. persica	0	0	0	0	.....	.....	.....	.....	.....	.....
P. serotina	0	0	0	0	.....	.....	.....	.....	.....	.....

[a] See footnote a, Table II.
[b–f] See footnotes b to f, Table I.
[g] See footnote g, Table II.
[h] Secondary infection was very abundant.

DISCUSSION OF TABLE IV

All the inoculations with strains from *P. mahaleb* induced either moderate or abundant infection. *P. cerasus*, *P. avium*, and *P. mahaleb* were consistently moderately or abundantly infected. The disease was usually slightly less severe on *P. mahaleb* than on the cultivated cherries. This may well be due to the fact that these strains in all likelihood originally passed to *P. mahaleb* from *P. cerasus*, inasmuch as they were isolated from *P. mahaleb* trees which had grown from *P. cerasus* stocks in an old neglected orchard in Door County, where *P. mahaleb* is rarely found. *P. pennsylvanica* was also consistently infected, but less abundantly. In one case *P. besseyi* was infected out of doors. In certain cases, after prolonged incubation in the greenhouse, *P. insititia* and *P. munsoniana* developed infection, while in certain instances spotting occurred on *P. munsoniana*, *P. padus*, *P. domestica*, and *A. persica*. In one case in the greenhouse, flecks developed on *P. americana* B. No other infection occurred.

TABLE V.—*Summary of inoculation experiments with Coccomyces spp. from Prunus pennsylvanica, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination.[b]		
						After hours.	Per cent.	Vigor.
Out of doors:				*Months.*				
1B	June 8	Ascospores	Leaves, Sturgeon Bay.	.....	Dehiscence.	.....	.....	.....
6	July 24	Conidia	9-day-old culture	1.7	Spray	20	5	—
6	Aug. 4	do	8-day-old culture	2.1	do	20	5	—
12	July 31	do	10-day-old culture	2.0	do	17	5	+
12	Aug. 15	do	do	2.4	do	24	1	—
In greenhouse:								
107	May 22	do	do	8.6	do	48	−1	—
107	June 9	Ascospores	Leaves, Sturgeon Bay.	.....	Dehiscence.	.....	.....	.....
107	June 30	Conidia	7-day-old culture	.9	Spray	15	75	++
107	July 13	do	13-day-old culture	1.4	do	18	95	++
107	July 24	do	8-day-old culture	1.7	do	20	5	—
109	May 29	Ascospores	Leaves, Sturgeon Bay.	.....	Dehiscence.	40	80	+
109	June 12	Conidia	*P. pennsylvanica* leaves, series 109.	.....	Spray	48	1	—
109	June 28	do	*P. mahaleb* leaves, series 109.	.....	do	24	50	+

[a] See footnote a, Table II.
[b] See footnote b, Table I.

TABLE V.—*Summary of inoculation experiments with Coccomyces spp. from Prunus pennsylvanica, Madison, Wis., 1917*—Continued

RESULTS

Plant and treatment.	Infection.[c, g]												
	Out of doors.					In greenhouse.							
	Series 1B.	Series 6 from inoculation of—		Series 12 from inoculation of—		Series 107 from inoculation of—					Series 109 from inoculation of—		
		July 24.	August 4.	July 31.	August 15.	May 22.	June 9.	June 30.	July 13.	July 24.	May 29.	June 12.	June 28.
Inoculated:													
P. cerasus	.....	0	0	f3	e0	0	0	0	d0	0	0	0	0
P. avium	.....	.....	.....	3	3	.....	.....	.....	.....	.....	.....	.....	.....
P. mahaleb	.....	.....	.....	2	0	0	4	3	.....	2	3	4	4
P. pennsylvanica	5	3	5	4	3	0	5	.....	3	2	5	5	4
P. serotina A	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. serotina B	.....	.....	.....	0	0	.....	.....	.....	.....	.....	0	0	0
P. serotina C	.....	.....	.....	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. padus	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0
P. domestica	.....	0	0	0	0	0	0	0	0	d0	0	0	0
P. insititia	.....	0	0	0	0	0	0	0	0	d0	0	e0	0
P. americana A	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. americana B	.....	0	0	0	0	0	0	0	0	d0	0	0	0
P. salicina	.....	0	0	0	0	0	0	0	0	0	0	0	0
P. munsoniana	.....	0	0	0	0	0	0	0	0	d0	(?)	f2	0
A. persica	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. armeniaca	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. besseyi	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. cerasifera (*P. domestica* stock)	.....	.....	.....	.....	.....	.....	.....	(?)	(?)	f3	.....	.....	.....
Not inoculated:													
P. pennsylvanica	0	0	0	0	0	0	0	0	0	0	0	0	.....
P. serotina B	.....	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. serotina C	.....	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
P. domestica	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. armeniaca	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. americana B	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
A. persica	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. cerasus	.....	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....
P. virginiana	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

c-f See footnotes c to f, Table I. *g* See footnote g, Table II.

DISCUSSION OF TABLE V

The first inoculation of series 107 induced no infection. This was evidently due to the unsatisfactory nature of the inoculum, as is witnessed by its low percentage of germination. This inoculum was secured from a strain that had been more than eight months in culture. The last inoculation of this series caused less abundant infection than was expected. The cause of this fact is not understood. It may possibly be correlated with the high temperatures that developed in the greenhouse in midsummer. All other inoculations with strains from *P. pennsylvanica* caused either moderate or abundant infection. *P. pennsylvanica*, *P. mahaleb* (except in the second inoculation of series 12), and *P. avium* were consistently infected. In one case, after a prolonged incubation period, *P. cerasus* was infected. A single case of delayed infection like-

wise occurred on *P. munsoniana*. The single plant tested of *P. cerasifera* developed fairly abundant delayed infection. No other infection was observed. In certain cases, however, usually in the greenhouse, flecks or spots occurred on *P. cerasus*, *P. domestica*, *P. insititia*, *P. americana* B, and *P. munsoniana*.

TABLE VI.—*Summary of inoculation experiments with Coccomyces spp. from Prunus domestica, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination.[b]		
						After hours.	Per cent.	Vigor.
Out of doors:				*Months.*				
5	July 23	Conidia	8-day-old culture	1.6	Spray	24	15	+
5	Aug. 28	do	do	2.9	do	36	80	—
16	Sept. 20	do	14-day-old culture	1.8	do	17	5	+
In greenhouse:								
103	May 14	do	11-day-old culture	20.5	do	20	60	+
103	June 14	do	27-day-old culture	21.5	do	.....	.....	.....
103	June 30	do	15-day-old culture	.9	do	15	50	+
110	June 1	Ascospores	Leaves, Sturgeon Bay.	.....	Dehiscence	24	80	+
110	June 30	Conidia	15-day-old culture	.9	Spray	15	50	+
110	July 15	do	do	1.3	do	24	75	+

RESULTS

Plant and treatment.	Infection.[c, g]								
	Out of doors.			In greenhouse.					
	Series 5 from inoculation of—		Series 16.	Series 103 from inoculation of—			Series 110 from inoculation of—		
	July 23.	Aug. 28.		May 14.	June 14.	June 30.	June 1.	June 30.	July 15.
Inoculated:									
P. cerasus	0	0	0	0	0	0	0	0	.....
P. mahaleb	0	0	.....	0	0	[f] 3	0	1	1
P. pennsylvanica	0	0	.....	.....	.....	.....	.....	.....	.....
P. serotina A	0	0	.....	.....	.....	.....	.....	.....	.....
P. serotina B	0	0	0	0	0	0	0	0	0
P. serotina C	0	0	.....	.....	.....	.....	.....	.....	.....
P. padus	[d] 0	[d] 0	0	.....	.....	.....	.....	.....	.....
P. virginiana	0	0	.....	0	0	0	0	0	0
P. domestica	4	4	4	2	2	5	[h] 0	5	.....
P. insititia	4	4	4	1	2	5	3	5	.....
P. americana A	4	2	2	.....	.....	.....	.....	.....	.....
P. americana B	4	2	4	0	0	4	3	5	.....
P. salicina	[e] 0	[e] 0	0	0	0	[f] 4	.....	.....	.....
P. munsoniana	0	0	0	0	0	[f] 4	0	0	[e] 0
A. persica	0	0	.....	0	0	[f] 1	(?)	[f] 1	.....
P. armeniaca	0	0	.....	.....	.....	.....	.....	.....	.....
P. besseyi	(?)	[f] 1	.....	.....	.....	.....	.....	.....	.....
Not inoculated:									
P. domestica	0	0	0	.....	.....	.....	0	0	0
P. americana B	0	0	.....	0	0	0	.....	.....	.....
P. cerasus	0	0	.....	.....	.....	.....	.....	.....	.....
A. persica	0	0	.....	.....	.....	.....	.....	.....	.....
P. armeniaca	0	0	.....	.....	.....	.....	.....	.....	.....
P. insititia	.....	.....	0	.....	.....	.....	.....	.....	.....
P. serotina B	0	0	0	.....	.....	.....	.....	.....	.....

[a] See footnote a, Table II.
[g] See footnote g, Table II.
[b–f] See footnotes b to f, Table I.
[h] Failure probably due to some local defect in manipulation of inoculation

DISCUSSION OF TABLE VI

Either a moderate or an abundant infection (Pl. 57) resulted from all inoculations except the first and second of series 103 and the third of series 110. The sparseness of infection in series 103 was probably due to a diminution of the pathogenicity of the fungus in culture, as both of the tests were made with inocula from a strain which had been in culture for more than 20 months. The reasons for the low degree of infection from the last inoculation of series 110 are not understood. It is possible again that the high temperatures of this period may have been inhibitory. *P. domestica* (see footnote h), *P. insititia*, and *P. americana* were consistently infected. *P. mahaleb* was sparsely infected, but not consistently. *P. salicina* and *P. munsoniana*, in one case each, developed infection after prolonged incubation in the greenhouse. Similarly, in two cases, *A. persica* developed sparse infection. The single test with *P. besseyi* yielded positive results after prolonged incubation. No other infection was observed. Sometimes spots or flecks developed on *P. padus*, *P. salicina*, and *P. munsoniana*.

TABLE VII.—*Summary of inoculation experiments with Coccomyces spp. from Prunus virginiana, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination. [b]		
						After hours.	Per cent.	Vigor.
Out of doors:				*Months.*				
1C	June 8	Ascospores	Leaves, Madison	.....	Dehiscence	.....	.....	.....
4	July 21	Conidia	*P. virginiana* leaves, series 113 and 117.	.....	Spray	48	− 1	−
4	Aug. 14	....do	9-day-old culture	1.6	....do	24	0	.....
13	Aug. 2	....do	*P. virginiana* leaves, series 117 and 119.	.....	....do	24	− 1	−
13	Aug. 21	....do	12-day-old culture	2.6	....do	.....	.....	.....
In greenhouse:								
111	June 8	Ascospores	Leaves, Sturgeon Bay.	.....	Dehiscence	48	95	+
111	July 13	Conidia	13-day-old culture	1.3	Spray	18	5	+
111	July 28	....do	*P. virginiana* fruit, Two Rivers, Wis.	.....	....do	24	− 1	−
113	June 26	....do	21-day-old culture	.7	....do	24	0	.....
113	July 10	....do	*P. virginiana* leaves, series 113.	.....	....do	48	25	+
117	July 2	....do	15-day-old culture	.9	....do	48	− 1	−
117	July 23	....do	8-day-old culture	1.6	....do	.....	.....	.....
119	July 10	....do	10-day-old culture	1.2	....do	48	0	.....
119	July 23	....do	8-day-old culture	1.6	....do	24	− 1	−
121	July 31	....do	*P. virginiana* leaves, series 108. [j]	.....	....do	17	50	+

[a] See footnote a, Table II.
[b] See footnote b, Table I.
[j] See Table VIII.

TABLE VII.—*Summary of inoculation experiments with Coccomyces spp. from Prunus virginiana, Madison, Wis., 1917*—Continued

RESULTS

Plant and treatment.	Infection. [c, g]														
	Out of doors.					In greenhouse.									
	Series 1C.	Series 4, from inoculation of—		Series 13, from inoculation of—		Series 111, from inoculation of—			Series 113, from inoculation of—		Series 117, from inoculation of—		Series 119, from inoculation of—		Series 121.
		July 21.	Aug. 14.	Aug. 2.	Aug. 21.	June 8.	July 13.	July 28.	June 26.	July 10.	July 2.	July 23.	July 10.	July 23.	
Inoculated:															
P. cerasus	....	0	0	0	0	0	[i](?)	0	0	0	0	0	0	0	....
P. mahaleb	....	....	....	....	*f*1	2	1	0	*f*2	*f*2	0	*f*1	1	2	[e]0
P. pennsylvanica	0	0	0	0	0	....	....	....	....	....	0	[e]0	....	....	....
P. serotina A	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
P. serotina B	....	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. padus	....	[h]3	[h]3	[h]3	[h]3	....	....	....	....	....	....	....	....	....	....
P. virginiana	5	2	3	[h]4	[h]4	....	....	2	5	5	2	5	1	5	4
P. domestica	....	0	0	0	*f*2	4	*f*2	0	*f*3	*f*3	....	....	*f*2	*f*4	....
P. insitilia	....	0	0	....	*f*2	1	1	0	....	....	....	....	*f*1	*f*3	....
P. americana A	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
P. americana B	....	0	0	0	0	0	0	0	....	....	....	....	....	....	....
P. salicina	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....
P. munsoniana	....	0	0	0	0	0	0	0	....	....	....	....	....	....	....
A. persica	....	0	0	0	0	....	....	....	....	....	....	....	0	0	....
P. armeniaca	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....
P. besseyi	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
P. mahaleb (*P. cerasus* stock)	....	....	....	....	....	....	....	....	*f*3	*f*2	....	....	....	....	....
Not inoculated:															
P. virginiana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	....
P. domestica	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
P. americana B	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
P. serotina	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
A. persica	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....
P. cerasus	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....
P. armeniaca	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....
P. mahaleb	....	....	....	0	0	....	....	....	....	....	....	....	....	....	....
P. pennsylvanica	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....

[c], [e], [f] See footnotes c, e, f, Table I.
[g] See footnote g, Table II.
[h] Secondary infection was very abundant.
[i] See discussion, p. 561.

DISCUSSION OF TABLE VII

Either moderate or abundant infection resulted from all inoculations except the second and third of series 111, the first of series 117, and the first of series 119. In all these cases the sparseness of infection was probably attributable to unsatisfactory inocula. It will be noted that all the inocula were obtained from cultures. Of all the strains of fungi used in these inoculation experiments, those from *P. virginiana* were by far the most difficult to induce to sporulate satisfactorily in culture. Thus, in certain cases the inocula were unsatisfactory because of sparseness of spores. It will also be observed that the inocula of Table VII usually germinated less vigorously than did those of the other tables. Negative germination results in the laboratory tests,

however, do not necessarily prove that inocula are ineffective, as is attested by the results of the second inoculation of series 4.

P. virginiana (Pl. 59, E), *P. padus*, and *P. mahaleb* (except in series 121) were consistently infected. *P. mahaleb*, however, was uniformly less severely diseased than the other species named. *P. domestica* and *P. insititia* were frequently infected, usually after prolonged incubation. On August 3 several leaves of *P. cerasus* of series 111 bore scattered brown spots on which occurred numerous aggregated acervuli of a species of Cylindrosporium. It is uncertain whether this was the result of chance infection or of delayed infection. The fact that no infection occurred on the extensive control system makes the possibility of chance infection remote. However, the fact that 10 other inoculations of *P. cerasus* with strains from *P. virginiana* gave uniformly negative results is a strong argument against fully accepting this result as positive. Furthermore, the fungus when isolated possessed the cultural characters typical of strains from *P. cerasus*, which were very different from those from *P. virginiana*. Further experiments will be necessary before conclusions regarding this cross are justifiable. Meanwhile this apparently aberrant result is not included in the summary (Table IX). No other infection was observed.

TABLE VIII.—*Summary of inoculation experiments with Coccomyces spp. from Prunus serotina, Madison, Wis., 1917*

INOCULATIONS [a]

Location and series.	Date.	Inoculum.						
		Spores.	Source.	Age of strain in culture.	Method of application.	Germination.[b]		
						After hours.	Per cent.	Vigor.
Out of doors:				*Months.*				
1D	June 8	Ascospores	Leaves, Madison	.....	Dehiscence	.....	.....	.....
3	July 19	Conidia	9-day-old culture	1.7	Spray	24	80	—
3	Aug. 13	...do	...do	2.5	...do	17	— 1	—
9	July 27	...do	*P. serotina* leaves, series 105.	.....	...do	24	60	+
9	Sept. 1	...do	12-day-old culture	3.0	...do	36	5	+
19	Sept. 24	...do	17-day-old culture	1.9	...do	24	15	+
In greenhouse:								
105	May 18	...do	8-day-old culture	8.2	...do	48	— 1	—
105	June 6	Ascospores	Leaves, Madison	.....	Dehiscence	.....	.....	.....
105	June 13	...do	...do	.....	Spray[i]	48	— 1	—
105	June 29	Conidia	20-day-old culture	1.1	Spray	18	50	—
105	July 13	...do	13-day-old culture	1.5	...do	18	15	+
108	May 24	Ascospores	Leaves, Madison	.....	Spray[i]	.....	.....	.....
108	June 3	...do	...do	.....	Spray[h]	24	50	+
108	July 4	Conidia	21-day-old culture	1.2	Spray	.....	.....	.....
108	July 23	...do	8-day-old culture	1.8	...do	.....	.....	.....
112	June 19	...do	6-day-old culture	1.0	...do	24	2	+
112	July 4	...do	21-day-old culture	1.2	...do	.....	.....	.....
120	July 17	...do	*P. mahaleb* leaves, series 105.	.....	...do	48	— 1	—
120	July 28	...do	*P. mahaleb* leaves, series 120.	.....	...do	20	10	—

[a] See footnote a, Table II.
[b] See footnote b, Table I.
[h] See discussion, p. 563.
[i] Crushed as cocarps in sterile distilled water.

TABLE VIII.—*Summary of inoculation experiments with Coccomyces spp. from Prunus serotina, Madison, Wis., 1917*—Continued

RESULTS

Plant and treatment.	Infection. [c, g]																		
	Out of doors.						In greenhouse.												
		Series 3 from inoculation of—		Series 9 from inoculation of—			Series 105 from inoculation of—					Series 108 from inoculation of—				Series 112 from inoculation of—		Series 120 from inoculation of—	
	Series 1D.	July 19.	Aug. 13.	July 27.	Sept. 1.	Series 19.	May 18.	June 6.	June 13.	June 29.	July 13.	May 24.	June 3.	July 4.	July 23.	June 19.	July 4.	July 17.	July 28.
Inoculated:																			
P. cerasus	....	h(?)	0	0	0	0	0	0	0	0	0	0	0	(?)	h(?)	0	0	0	0
P. mahaleb	....	2	2	0	0	....	0	1	1	2	....	....	....	....	....	0	1	1	0
P. pennsylvanica	0	0	0	....	....	0	....	....	....	....	....	....	....	....	....	0	....	....	....
P. serotina A	....	5	5	4	3	....	....	....	....	....	....	....	....	....	....	....	....	....	....
P. serotina B	....	5	5	2	2	5	0	3	2	4	5	2	5	4	2	4	4	3	2
P. padus	....	e 0	e 0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
P. virginiana	0	0	0	0	0	....	0	0	(?)	(?)	h(?)	0	0	(?)	h(?)	0	0	....	....
P. domestica	....	0	0	0	0	0	0	0	0	0	0	....	....	....	....	....	....	....	....
P. insititia	....	0	0	0	0	0	0	0	0	f 4	f 4	....	....	....	....	....	....	....	....
P. americana A	....	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
P. americana B	....	0	0	0	0	0	0	0	0	0	0	....	....	....	....	....	....	....	....
P. salicina	....	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
P. munsoniana	....	0	0	0	0	0	0	0	0	0	0	....	....	....	....	....	....	....	....
A. persica	....	0	0	0	0	0	0	0	0	0	0	....	....	....	....	....	....	....	....
P. armeniaca	....	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
P. besseyi	....	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....
P. mahaleb (*P. cerasus* stock)	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	2
Not inoculated:																			
P. serotina B	....	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. virginiana	0	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
P. domestica	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
P. americana B	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
P. cerasus	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
A. persica	....	0	0	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....
P. pennsylvanica	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
P. avium	....	0	0	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

[c, e, f] See footnotes c, e, f, Table I. [g] See footnote g, Table II. [h] See discussion, p. 563.

DISCUSSION OF TABLE VIII

Either moderate or abundant infection resulted from all inoculations with strains from *P. serotina*, except the first and third of series 105 and the first and fourth of series 108. The failure of the first test on series 105 was evidently due to an unsatisfactory inoculum, as is witnessed by its low germination and the age of the strain in culture (8.2 months). The sparseness of infection from the third test on series 105 and the first on series 108 is clearly due, likewise, to unsatisfactory inocula. In both of these tests the inocula were prepared by crushing fresh ascocarps in sterile distilled water, a method which was subsequently discarded as unsatisfactory. The reasons for the sparseness of infection from the last inoculation of series 108 are not understood. They may well be associated with the high temperatures which prevailed in the greenhouses at the time of this test.

Of all the experimental plants, only *P. serotina* (Pl. 58, A) was consistently and abundantly infected. *P. mahaleb* (Pl. 58, B) was usually infected, though ordinarily rather sparsely . In two instances, after prolonged incubation in the greehouse, *P. insititia* (Pl. 58, C) developed abundant infection. On July 30, eleven days after inoculation, two of of the older leaves of *P. cerasus* of series 3 each bore a small group of closely aggregated lesions, which appeared to be secondary infections from a single primary infection on each leaf. These leaves were collected, and no further infection had developed when the plant was last noted, on October 15. Although no infection developed on the elaborate control system of the outdoor experiments, the writer believes this to be a case of chance infection. It is the only case found in the outdoor series. The bed was the one nearest to the pathologium (about 35 feet away) in which all the greenhouse inoculations were made; and it seems probable that this infection resulted from wind-borne ascospores or particles of atomized spore suspensions from the pathologium. This result is, therefore, not included in Table IX.

On August 3 one leaf of *P. virginiana* of series 105 was observed to bear numerous, closely aggregated infections. On August 7 three other leaves on the same plant and one leaf of *P. virginiana* of series 108 were found to be similarly infected. Likewise on August 3 several leaves of *P. cerasus* of series 108 were observed to bear dead brown patches on which were numerous aggregated acervuli of a species of Cylindrosporium. It is uncertain whether these results were due to a chance infection or to delayed infection. The freedom of all control plants (Pl. 58, D) from infection, and the fact that in all the greenhouse series no other such questionable cases occurred, are strong arguments against accepting the theory of chance infection. Upon the other hand, the fact that 11 similar tests on *P. cerasus* and 9 on *P. virginiana* uniformly failed to induce infection makes one hesitate to conclude that this infection resulted from the inoculations. These questions can be definitely settled only by further experiments. Meanwhile these three questionable cases are not included in the summary (Table IX). No other infection was observed.

REISOLATIONS

While it was deemed neither feasible nor necessary to make reisolations from all the crosses effected, large numbers of reisolations were made from both the outdoor and the greenhouse inoculations. Especial attention was given to reisolating from difficult crosses. Although the slow growth of the fungi in culture made this work slow and laborious, no serious difficulty was experienced in making reisolations wherever the fungi fructified with fair vigor. It was important, however, to choose the best possible material from which to isolate. Each reisolated strain agreed closely in cultural characters with the strain from which the inoculation in question had been made.

TABLE IX.—*Summary of results of inoculation experiments with Coccomyces spp. from Prunus spp., Madison, Wis., 1916 and 1917* [a]

[The average degree of infection is represented by the average of the numbers representing the severity of infection from the individual inoculations which gave positive results (see footnote c, Table I). The results of series in which the maximum infection is represented by a number less than 3 and those which represent the combined infection from two or more inoculations (see footnote j, Table II) are not included].

Plant inoculated.	Results with strains from—																				
	P. cerasus.			*P. avium.*			*P. mahaleb.*			*P. pennsylvanica.*			*P. domestica.*			*P. virginiana.*			*P. serotina.*		
	Number positive.[b]	Number negative.	Average degree of infection.	Number positive.[b]	Number negative.	Average degree of infection.	Number positive.[b]	Number negative.	Average degree of infection.	Number positive.[b]	Number negative.	Average degree of infection.	Number positive.[b]	Number negative.	Average degree of infection.	Number positive.[b]	Number negative.	Average degree of infection.	Number positive.[b]	Number negative.	Average degree of infection.
P. cerasus	9	0	4.1	7	0	3.4	11	0	3.9	1	9	3.0	0	6	....	0	10	....	0	11	....
P. avium	[c]1	0	5.0	[c]1	0	4.0	4	0	4.7	[c]2	0	3.0	(c)	...	....	0	[c]2	....	(c)	...	....
P. mahaleb	12	[d]1	3.2	6	0	3.0	13	0	2.7	7	1	3.3	3	3	2.0	11	1	2.0	10	3	1.4
P. pennsylvanica	0	[c]2	....	[c]0	3	....	4	1	2.5	11	0	4.2	[c]0	2	....	0	7	....	0	5	....
P. serotina	0	11	....	0	9	....	0	16	....	0	11	....	0	10	....	0	16	....	21	0	4.0
P. padus	0	2	....	0	3	....	0	5	....	0	4	....	0	3	....	4	0	3.0	0	5	....
P. virginiana	0	9	....	0	5	....	0	8	....	0	11	....	0	5	....	14	0	4.2	0	9	....
P. domestica	0	9	....	0	5	....	0	11	....	0	10	....	8	[d]1	4.4	7	4	3.2	0	8	....
P. insititia	[e]4	3	3.5	0	6	....	[e]2	7	....	0	10	....	9	0	4.2	6	2	2.0	[e]2	6	4.0
P. americana	0	12	....	0	10	....	0	17	....	0	14	....	9	0	3.3	0	11	....	0	13	....
P. salicina	0	9	....	0	6	....	0	4	....	0	10	....	[e]1	3	4.0	0	3	....	0	5	....
P. munsoniana	4	2	3.3	0	6	....	[e]3	7	2.5	[e]1	8	....	[e]1	5	4.0	0	6	....	0	8	....
P. cerasifera	[e]1	0	....	...	...	....	...	...	....	[e]1	0	....	...	...	....	...	...	....	...	...	....
A. persica	0	6	....	0	6	....	0	11	....	0	4	....	[e]2	2	1.0	0	5	....	0	8	....
A. persica nectarina	0	1	....	0	1	....	0	1	....	...	...	....	...	...	....	0	1	....	...	...	....
P. armeniaca	0	[c]1	....	0	3	....	0	5	....	0	4	....	0	2	....	0	3	....	0	5	....
P. besseyi	1	2	4.0	1	3	4.0	1	5	3	0	4	....	1	0	....	0	5	....	0	5	....

[a] Compiled from Tables I–VIII.
[b] All inoculations in which the fungus induced lesions and fructified are listed as positive. Cases of flecking and spotting without fructification are listed as negative. Cases in which the results of successive inoculations on the same plant were indistinguishable are treated as single inoculations.
[c] The number of experiments was limited by the failure of experimental plants to take root.
[d] Inoculated by ascospores applied by natural discharge. See discussion, p. 550.
[e] Positive results were obtained from greenhouse experiments only.

DISCUSSION OF RESULTS

A perusal of Table IX, which contains a summary of the results of the experiments of 1916 and 1917 will show that in no case did the strains from any two host plants give identically the same results. The results from strains from *P. cerasus*, *P. avium*, and *P. mahaleb* differ only very slightly, however, and from the standpoint of host relationships the strains from these species may clearly be grouped together. The strains from *P. cerasus* readily and consistently infected *P. cerasus*, *P. avium*, and *P. mahaleb*, while in certain cases, and apparently with difficulty, they infected *P. insititia*, *P. munsoniana*, *P. cerasifera*, and *P. besseyi*. The strains from *P. mahaleb* induced infection on all the plants just mentioned, except *P. cerasifera*, upon which they were not tested. In addition, however, they also infected *P. pennsylvanica*. This is the only important regard in which their results differed from those of strains from *P. cerasus* and *P. avium*. It must be borne in mind, however, that, owing to the failure of experimental plants to root, the number of tests with strains from *P. cerasus* and *P. avium* on *P.*

pennsylvanica was very limited, and it is possible that further trials may yield positive results. The results from the strain from *P. avium* paralleled those from the *P. cerasus* strains, with the exception that no infection was induced on plums. It should be remembered, however, that only one strain from *P. avium* was available, and its pathogenicity appeared to have been diminished in culture.

The strains from *P. pennsylvanica* infected *P. avium*, *P. mahaleb*, and *P. pennsylvanica* abundantly, and *P. cerasus*, *P. munsoniana*, and *P. cerasifera* with difficulty. They differed in behavior from those from *P. cerasus* chiefly in their abundant infection of *P. pennsylvanica* and their sparse infection of *P. cerasus*, on which but 1 trial of 10 gave positive results. It is important to note, however, that, while only one cross from *P. pennsylvanica* to *P. cerasus* was effected, and the reverse cross has not yet been made, crosses between *P. pennsylvanica* and *P. mahaleb*, on the one hand, and *P. cerasus* and *P. mahaleb*, on the other, were readily effected. Furthermore, *P. pennsylvanica* readily infected *P. avium*, which easily cross-infects with *P. cerasus*. It appears, therefore, that while the results from strains from *P. pennsylvanica* do not accord perfectly with those from strains secured from *P. cerasus*, *P. avium*, and *P. mahaleb*, the agreement is so close that, from the standpoint of host relationships, the strains from these four species may tentatively be considered as a single group in which varying degrees of specialization have been developed. It remains, however, for further experiments to define the extent and the constancy of this specialization. Attention is called to the fact that, while the crosses just reported suggest the possibility that strains from *P. pennsylvanica* may pass readily to *P. cerasus* by way of *P. mahaleb* or *P. avium*, no experiments were made in which a given strain was carried over in this manner. Such experiments are projected. Further discussion of these problems of specialization and a consideration of the specific relationships of the various strains of Coccomyces used in these experiments are reserved for a later paper.

The results from strains taken from *P. domestica*, *P. virginiana*, and *P. serotina* are so different that, from the standpoint of host relationships, no further grouping of strains seems feasible. Strains from *P. domestica* infected *P. domestica* and *P. americana* abundantly, and *P. salicina*, *P. munsoniana*, *A. persica*, *P. besseyi*, and *P. mahaleb* with difficulty. Those from *P. virginiana* infected *P. virginiana* and *P. padus* easily and abundantly, and *P. mahaleb* consistently (except one trial), but uniformly less abundantly. They infected *P. domestica* and *P. insititia* with difficulty. The strains from *P. serotina* showed a high degree of specialization. They infected *P. serotina* uniformly and abundantly, *P. mahaleb* fairly consistently but sparsely, and *P. insititia* with difficulty.

A condensed summary of the results of all the crosses tried appears in Table X. Of all the experimental plants, *P. mahaleb* showed the widest range of susceptibility, being infected by inocula from all the host sources

tested—viz, *P. cerasus*, *P. avium*, *P. mahaleb*, *P. pennsylvanica*, *P. domestica*, *P. virginiana*, and *P. serotina*. Details regarding the relative consistency and abundance of the infection induced in these crosses are found in Table IX. *P. cerasus* and *P. avium* were infected by inocula from *P. cerasus*, *P. avium*, *P. mahaleb*, and *P. pennsylvanica*, though in the case of *P. cerasus* the cross from *P. pennsylvanica* was effected but once in 10 trials. *P. pennsylvanica* showed a narrower range of susceptibility, being infected by inocula from *P. pennsylvanica* and *P. mahaleb* only. It should be remembered, however, that this cross was tried but twice with strains from *P. cerasus* and three times with inocula from *P. avium* (see footnote d, Table X). *P. serotina* and *P. virginiana*, respectively, were infected only by their own strains. *P. padus* was infected only by strains from *P. virginiana*. The results with *P. padus* are of special interest, inasmuch as this is the host upon which Karsten [1] originally described *Cylindrosporium padi*.

TABLE X.—*Summary of results of crosses tried in inoculation experiments with Coccomyces spp. from Prunus spp., Madison, Wis., 1916 and 1917*

Plants inoculated.	Results [a] with strains from—						
	P. cerasus.	*P. avium.*	*P. mahaleb.*	*P. pennsylvanica.*	*P. domestica.*	*P. virginiana.*	*P. serotina.*
P. cerasus	+	+	+	[c] +	−	−	−
P. avium	[d] +	[d] +	+	[d] +	.....	[d] −	.....
P. mahaleb	+	+	+	+	+	+	+
P. pennsylvanica	[d] −	[d] −	+	+	[d] −	−	−
P. serotina	−	−	−	−	−	−	+
P. padus	−	−	−	−	−	+	−
P. virginiana	−	−	−	−	−	+	−
P. domestica	−	−	−	−	+	+	−
P. insititia	[b] +	−	[b] +	−	+	+	[b] +
P. americana	−	−	−	−	+	−	−
P. salicina	−	−	−	−	[b] +	−	−
P. munsoniana	+	−	[b] +	[b] +	[b] +	−	−
P. cerasifera	[b] +	.....	.....	[b] +	.....	.....	.....
A. persica	−	−	−	−	[b] +	−	−
A. persica nectarina	−	−	−	.....	.....	−	.....
P. armeniaca	−	−	−	−	−	−	−
P. besseyi	+	+	+	−	+	−	−

[a] Compiled from Table IX. A single positive result serves to list the results of a cross as positive, even though a large number of tests may have given negative results. See footnote c.
[b] Positive results were obtained only after prolonged incubation in the greenhouse.
[c] This cross was effected but once in 10 trials.
[d] The number of experiments was limited, owing to the failure of experimental plants to root.

Of the plums tested, *P. insititia* showed the widest range of susceptibility, being infected readily by strains from *P. domestica*, fairly consistently though rather sparsely by strains from *P. virginiana*, and difficultly by inocula from *P. cerasus*, *P. mahaleb*, and *P. serotina*. *P. domestica* was infected consistently and abundantly by its own strains, and more difficultly by strains from *P. virginiana*. *P. americana* and *P. salicina* were infected only by inocula from *P. domestica*, the former abundantly

[1] KARSTEN, P. A. SYMBOLAE AD MYCOLOGIAM FENNICAM. PARS XVI. *In* Meddel. Soc. Fauna et Flora Fennica, Häftet 11, p. 159. 1885.

and consistently, and the latter with difficulty. *P. munsoniana* was infected more readily by strains from *P. cerasus* than those from any other source tried. While these strains induced fairly consistent and abundant infection a prolonged incubation period always resulted, and the cross appeared to be a rather difficult one. Infection on this host was induced with still greater difficulty by strains from *P. mahaleb*, *P. pennsylvanica*, and *P. domestica*. *P. cerasifera* was tested in only two instances (where sprouts developed from Lombard stocks). Delayed infection resulted in both cases, the inocula being, respectively, from *P. cerasus* and *P. pennsylvanica*. *A. persica* was infected difficultly and only by inocula from *P. domestica*. *A. persica nectarina* and *P. armeniaca* were tested only in the experiments of 1916, and with uniformly negative results. *P. besseyi* was difficultly infected by inocula from *P. cerasus*, *P. avium*, *P. mahaleb*, and *P. domestica*. The waxy cuticle of the leaves of this species is very resistant to wetting. This fact may be in some measure accountable for the inconsistency of results from the various tests on *P. besseyi*.

While, for purposes of summarization, it was deemed advisable to omit from Tables IX and X details regarding the flecking and spotting reported in Tables I to VIII, these apparently aberrant types of infection should be given due consideration in the interpretation of the results of these experiments. Although the confirmatory histological studies necessary to justify conclusions have not yet been completed, this flecking and spotting, in conjunction with the types of delayed infection into which they merged, make it appear that various gradations of infection may occur, ranging in all likelihood from mere penetration of the germ tube to the production of typical, sporulating lesions. This condition presents many interesting problems, and appears to offer an excellent opportunity for fundamental studies of specialization of parasitism and of the intimate relations of host and parasite. It is fully realized, however, that, while more than a thousand inoculation tests have been made, these problems are little more than defined. For their material advancement much more extensive experiments will be necessary. These should include tests in which individual strains of the fungi are carried through many generations with the aim of determining, if possible, whether experimental changes in host relations induce detectable changes in parasitism. Such studies would bear upon the possibilities of variation among strains of different ancestry on a given host, and should include comparative tests of strains obtained from various sections. In this work due consideration should be given to the possibility of variations in the susceptibility of different varieties of host species and of individuals within a variety or species. Furthermore, as the greenhouse experiments have indicated, it would be of much interest and value to trace the effects of certain variable factors, particularly temperature and the vigor of the host plant, in relation to infection.

While final conclusions are not yet justifiable, the results of these experiments make it reasonably certain that under Wisconsin conditions the practical cherry grower need have no serious concern regarding infection of cultivated cherries by *Coccomyces* spp. from wild hosts, with the possible exception of *P. pennsylvanica*. So few sweet cherries are grown in the State that infection of *P. avium* from *P. pennsylvanica* is of little importance except as it relates to the possibility of infecting *P. cerasus* by way of the sweet cherry. The direct cross from *P. pennsylvanica* to *P. cerasus* was effected but once in 10 trials. It seems improbable, therefore, that such infection would be important. If *P. mahlaleb*, however, were of common occurrence within the State, it should be listed as a dangerous harborer of infectious material. In the case of the plums it is evident that the native *P. americana* may be a harborer of infectious material for cultivated species.

SUMMARY

This paper is a report of progress on more than 1,000 cross-inoculation tests with *Coccomyces* spp. from certain of the more common species of Prunus of Wisconsin.

From the standpoint of host relationships, the strains of fungi studied are tentatively grouped as follows, according to the hosts from which they were procured: (1) *P. cerasus*, *P. avium*, *P. mahaleb*, and *P. pennsylvanica*, (2) *P. domestica*, (3) *P. virginiana*, and (4) *P. serotina*. It is realized that minor variations in pathogenicity occur among strains within these groups.

The plants inoculated varied widely both in the range and the degree of their susceptibility to inocula from the various sources. *P. mahaleb* was notable for its wide range of susceptibility, being infected in varying degrees by inocula from all the host sources tested. Among the plums, *P. insititia* was notably susceptible, while the results of the two incidental inoculations of *P. cerasifera* suggest that it has likewise a wide range of susceptibility. *P. serotina* and *P. virginiana*, on the other hand, were notable for their resistance to cross infection, while *P. padus*, the host upon which Karsten[1] originally described *Cylindrosporium padi*, was infected only by strains from *P. virginiana*. *P. salicina*, *A. persica*, *P. armeniaca* and *A. persica nectarina* were notable for their resistance to all the strains tested.

While results are listed as positive only in cases where fructifying lesions were induced (p. 544), flecking and spotting without fungal fructification frequently resulted from inoculations on uncongenial hosts. It is tentatively assumed that such manifestations represent aberrant infection, all gradations of which probably occur.

[1] KARSTEN, P. A. LOC. CIT.

The variations of results in difficult crosses with conditions not yet fully understood make it unwise to place too strong reliance upon negative results.

It is apparent from these tests, though not fully conclusive, that in Wisconsin no serious infection of cultivated cherries is induced by inocula from wild hosts, with the possible exception of *P. pennsylvanica*. It is evident, however, that the native *P. americana* may act as a harborer of infectious material for cultivated plums.

This work has defined rather than solved, certain fundamental problems regarding host relationships and specialization of parasitism within the group of fungi under investigation.

PLATE 55

Prunus leaves from inoculation experiments, illustrating various degrees of infection, as recorded in Tables I to IX:

A.—*P. mahaleb*, infected by a strain of Coccomyces from *P. serotina*.

B.—*P. serotina*, infected by a strain from *P. serotina*.

C.—*P. cerasus*, infected by a strain from *P. avium*.

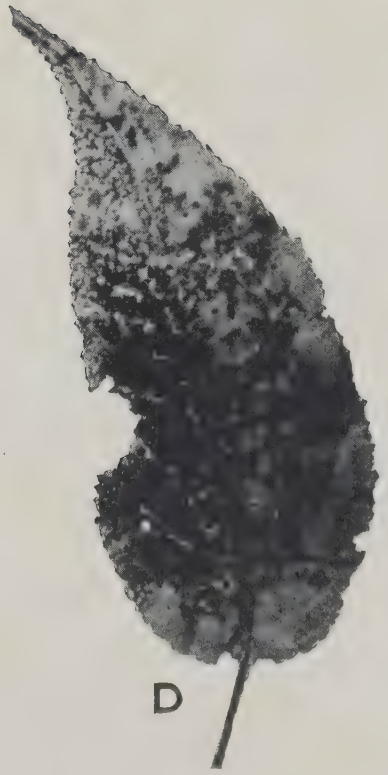
D.—*P. pennsylvanica*, infected by a strain from *P. pennsylvanica*.

Figures A, B, C, and D would be represented in the tables by the numbers 2, 3, 4, and 5, respectively. B approaches the maximum of 3, while C approaches the minimum of 4 (see footnote c, Table I).

A

B

C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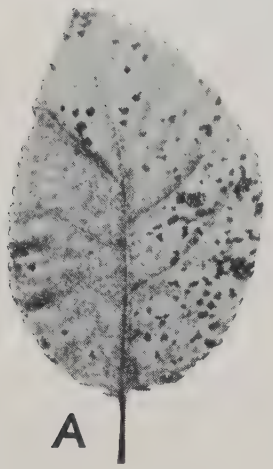


A

B

C

D

E

PLATE 56

Prunus leaves from inoculation series 104 (Table II), infected by strains of Coccomyces from *P. cerasus:*

A.—*P. cerasifera*, infected after prolonged incubation in the greenhouse.

B.—*P. insititia*, infected after prolonged incubation in the greenhouse.

C.—*P. mahaleb*.

D.—*P. munsoniana*, inoculated with naturally discharged ascospores on June 2; photographed on July 19. The infection appeared after prolonged incubation in the greenhouse.

E.—*P. domestica*. Spots developed after prolonged incubation in the greenhouse, but the fungus failed to fructify.

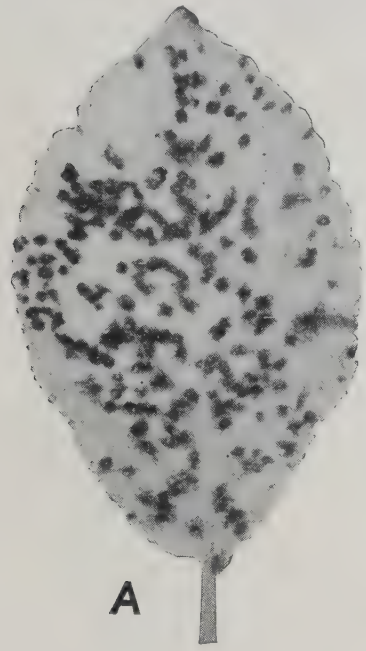
56113°—18——4

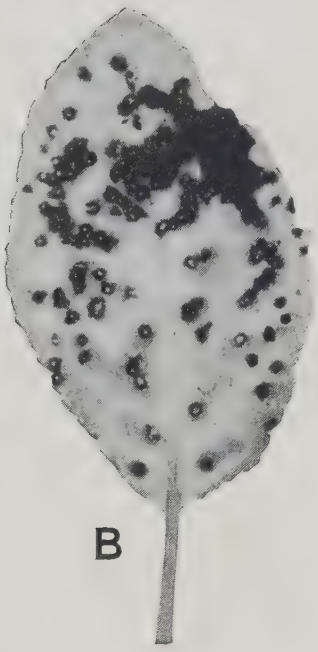
PLATE 57

Plum leaves from inoculation series 103 (Table VI):

A.—*P. domestica.*
B.—*P. insititia.*
C.—*P. domestica,* uninoculated.
D.—*P. americana.*
E.—*P. salicina.*

These leaves were inoculated comparatively with a strain of Coccomyces from *P. domestica.*


A

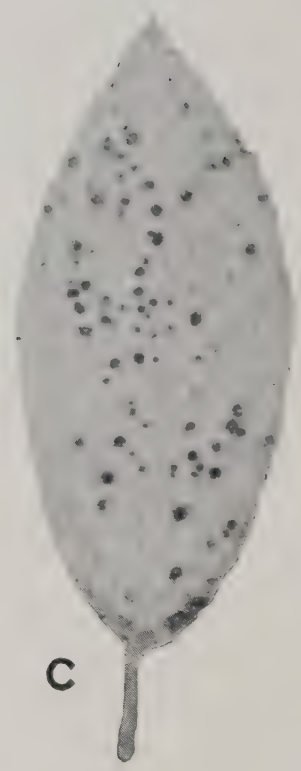

B

C

D

E

A



C

B

D

PLATE 58

Prunus leaves from inoculation experiments (Table VIII):

A.—*P. serotina*, infected by a strain of Coccomyces from *P. serotina*, series 3.

B.—*P. mahaleb*, sparsely infected by a strain from *P. serotina*, series 3.

C.—*P. insititia*, infected, after prolonged incubation in the greenhouse, by a strain from *P. serotina*, series 105.

D.—*P. serotina*, uninoculated, series 105.

PLATE 59.

Prunus leaves from inoculation experiments:

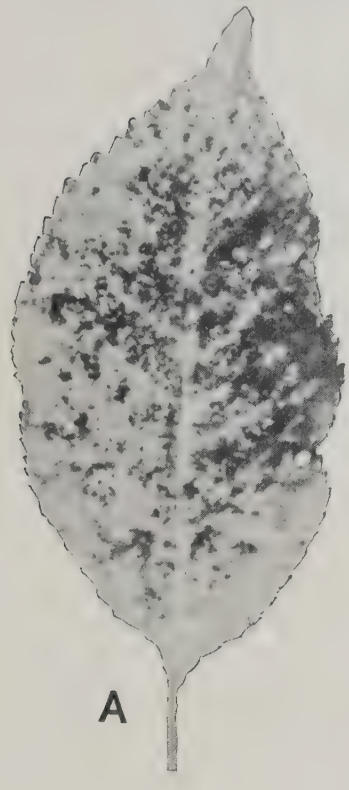
A.—*P. cerasus*, infected by a strain from *P. cerasus*, series 10 (Table II).

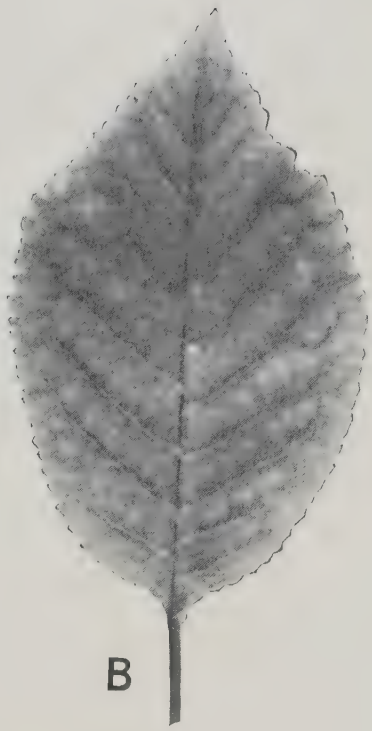
B.—*P. cerasus*, uninoculated, series 10.

C.—*P. pennsylvanica*, infected by naturally discharged ascospores from a leaf of *P. pennsylvanica*, series 1B (Table V).

D.—*P. cerasus*, infected by naturally discharged ascospores from a leaf of *P. cerasus*, series 101 (Table II).

E.—*P. virginiana*, infected by a strain from *P. virginiana*, series 13 (Table VII).


A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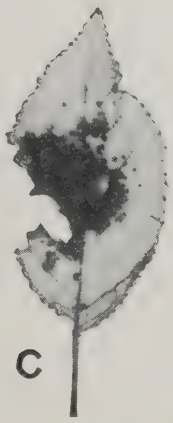
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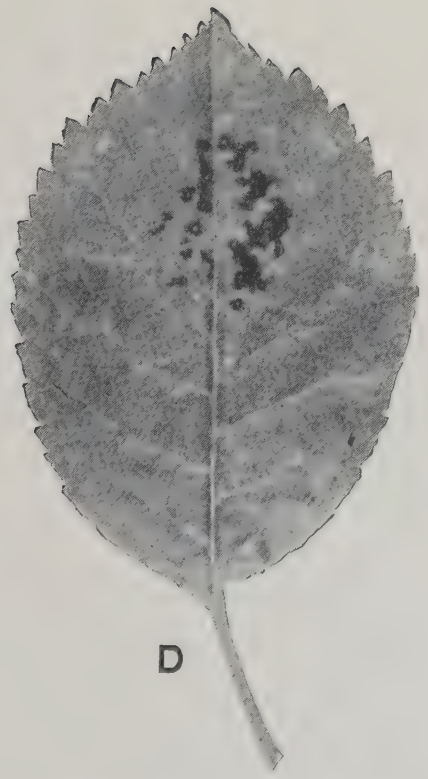
D

E

NYSIUS ERICAE, THE FALSE CHINCH BUG

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INTRODUCTION

The false chinch bug, *Nysius ericae* Schilling (*angustatus* Uhler), has been recognized for many years as a serious pest, especially in the semi-arid regions of the United States, where it causes great damage to sugar beets and cruciferous garden crops, settling upon them suddenly in enormous numbers and sucking so much sap from them that the plants wilt beyond recovery in one or two days.

When the writer was first stationed at Garden City, Kansas, in March, 1913, he could get no information regarding the life history and habits of the insect on which to base control measures. Work was therefore begun to determine these points, and the following account is prepared from data collected during that and the three following years.[1] The closest field study of the insect was made during 1913 and 1914, and the rearing work was done during 1914 and 1916.

DESCRIPTION

THE ADULT

The female is about 4 mm. long by 1.5 mm. wide. The greatest width is through the posterior edge of the prothorax and base of the wings. From this point the body tapers rapidly forward with a slight curve. The eyes project prominently on the sides at the posterior margin of the head, and the antennæ arise between the eyes and the base of the beak. The abdomen is elongate, its sides almost parallel and its apex rounded. It is entirely covered by clear membranous wings which project a little at the anal extremity. The ovipositor arises on the ventral surface of the tip of the abdomen, and is carried folded in a groove below the posterior abdominal segiments, the basal portion extending forward and the distal backward just beneath.

The males are perceptibly smaller than the females, or about half the length and half the width of a grain of wheat. Their form is similar to that of the female, excepting the tip of the abdomen, which is more pointed and without the groove on the venter.

The newly matured adult is dull whitish, but in a short time this changes to dirty gray with dark or black spots. Old adults (Pl. 60) are nearly black, except the ventral portions of the posterior abdominal segments of the female, which are gray or light brown. The wings remain transparent. The antennæ are uniform brownish, the legs and tarsi light brown with black spots, and the claws black.

[1] During the summers of 1914, 1915, and 1916 the writer was assisted by Mr. F. M. Wadley. Besides rendering assistance on the entire project, he alone collected the data for the topics, "Rate of oviposition at various hours of the day" and "Seasonal variation in oviposition."

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Schilling's original description, in Beiträge zur Entomologie, p. 86–87, Breslau, 1829, has been translated by Messrs. C. H. Popenoe and Gerson Garb as follows:

Heterogaster ericae. Grayish yellow; thoracic line transverse, hemelytra with discoidal punctures and posterior margin black. Membrane with smoky spots.

Lyg. thymi Fallen. Var. *a.*—Body smaller than in preceding; shape less narrow, lgt. 1¾ in., lt. ½ in. Head with black spots, median stripe pallescent. Antennæ dark, lighter at joints; thorax bears at the apex a line of black spots interrupted at the middle. The elytra cover the sides and apex of the abdomen; veins of hemelytra sprinkled with black punctures; membrane with indistinct smoky spots. Scutellum as in *thymi*. Abdomen beneath sprinkled with black, posteriorly with pale spots. Habitat on *Erica vulgaris* and allied plants.

THE EGG

FIG. 1.—*Nysius ericae:* Eggs, highly magnified.

The egg (fig. 1) is about 1.5 mm. long and 0.4 mm. wide, and tapers toward each end, one side being curved and the other nearly straight. It is of a translucent pinkish white color.

THE NYMPH

The body of the newly hatched nymph (Pl. 61, A) is about 0.7 mm. long by 0.3 mm. wide, and oval or pear-shaped, being widest behind the middle of the abdomen. Its color is translucent pinkish white, which on high magnification is seen to be due to irregular brownish opaque areas on an almost transparent background. The eyes are black and the segments of the antennæ shade almost to a flesh color at the distal ends. The dorsal portions of the fourth and fifth abdominal segments contain a large red mass which becomes indistinct after the first molt.

The nymphs become darker with age, but appear fresh and bright after each molt. After the first one (Pl. 61, B–E) there are dark areas on the sides of the thorax and anterior abdominal segments where the wing pads develop. These areas enlarge during the later instars and, with other sections of the body wall, become almost black.

The fifth instar (Pl. 61, E) is the pupa period. In it the insect is pear-shaped, and displays as much activity as is exhibited during earlier nymphal life.

LIFE HISTORY

OVIPOSITION

WHERE EGGS ARE PLACED.—The eggs are deposited in loose soil; among clods or rubbish; in composite flowers like the great-flowered gaillardia (*Gaillardia pulchella* Foug.); between the glumes in grasses like stink-grass or strong-scented love-grass (*Eragrostis major* Host.); and among the clustered parts of plants such as thyme-leaved spurge (*Chamaesyce serpyllifolia* Pers.), and carpet-weed (*Mollugo verticillata* L.); among the down from cottonwood (*Populus* spp.) wherever this down lodges in quantities; and in other similar places.

MANNER OF OVIPOSITION.—For egg-laying the female elevates the abdomen, straightens the ovipositor, and thrusts it almost vertically downward into the substance or among the parts chosen to receive the

eggs. Usually several trials are made before the female is satisfied and deposits the eggs, and sometimes the attempt is discontinued, to be resumed shortly afterwards.

Females have been observed to remain occupied at oviposition for as long as 9½ minutes, and as many as eight eggs have been found where a single female has been at work.

INCUBATION

Early in the incubation period a red spot appears near one end of the egg and at about the middle of the period two spots appear near the other end. The egg remains a translucent pink, and with proper magnification the developing nymph is plainly visible through the transparent shell.

NYMPHAL INSTARS

In conducting these experiments, after many unsuccessful attempts to rear the nymphs by ordinary laboratory methods, the newly-hatched nymphs were placed singly in bags of thin muslin or India linen which were slipped over the tips of growing plants and tied securely. This method proved more nearly successful than any other which was tried, though the handling necessary during examinations resulted in considerable loss through accidental injury to the nymphs or to their escape. At Garden City, Kans., in 1914, five individuals were held under close observation from hatching until death. At Wichita, Kans., in 1916, three individuals, one of which was kept until its death, were reared to maturity from eggs of known oviposition. The number which reached maturity, however, represents only about ½ of 1 per cent of the individuals used in the rearing experiments.

NUMBER AND LENGTH OF INSTARS

In Table I are given the number and length of instars obtained from five nymphs reared at Garden City in 1914.

TABLE I.—*Number and length of nymphal instars of Nysius ericae, Garden City, Kans., 1914*

Specimen No.	Number of days in each instar.						Length of nymphal life.	Sex.
	First.	Second.	Third.	Fourth.	Fifth.	Sixth.		
							Days.	
1	4	3	3	2	10	0	22	Male.
4	4	2	4	3	5	0	18	Do.
29	2	4	2	4	2	5	19	Female.
33	5	4	4	4	4	0	21	Male.
35	2	3	4	3	5	0	17	Do.

The only female reaching maturity (No. 29 in Table I) passed through six molts, aside from which nothing unusual was observed about her life cycle. There is slight chance for error, as the exuviæ in all cases were examined with a powerful magnifier.

Data were secured from other nymphs which remained under observation for only a portion of their life cycle, and during this period the average length of each instar was computed from these data on the basis of a much larger number. This is done in Table II which gives the average length of instars for the false chinch bug at Garden City.

TABLE II.—*Average length of nymphal instars of Nysius ericae, Garden City, Kans., 1914*

Instar.	First.	Second.	Third.	Fourth.	Fifth.
Number of nymphs	21	12	10	9	7
Days in instar (average)	4	3.75	3.2	3.1	6.3

Thus calculated, the length of the nymphal period is 20.35 days.

Data on the number and length of instars which were secured at Wichita during the rearing work of 1916 are presented in Table III.

TABLE III.—*Number and length of nymphal instars of Nysius ericae, Wichita, Kans., 1916*

Specimen No.	Number of days in each instar.					Sex.	Length of nymphal life.
	First.	Second.	Third.	Fourth.	Fifth.		
76	6	3	3	1	5	Female	18
149	7	7	3	5	15	Male	37
250	9	5	3	6	5	...do	28

Other data secured during 1916 permit averages to be presented for each instar with numbers as in Table IV, which gives the average length of instars for the false chinch bug at Wichita.

TABLE IV.—*Average length of nymphal instars of Nysius ericae, Wichita, Kans., 1916*

Instar.	First.	Second.	Third.	Fourth.	Fifth.
Number of nymphs	54	25	13	6	6
Days in instar (average)	5	3.76	3.6	4.5	3.83

This gives the length of the nymphal period as 20.69 days.

Tables I and III indicate great variation in the duration of the instars, both actually and relatively in proportion to the length of the entire nymphal period. The record of No. 29 in Table I indi-

cates a possible variation, also, in the number of instars. Further evidence of such variation was secured during 1916 from some males that apparently matured in 4 molts. Apparently 5 molts is normal; but the variation in the actual as well as the relative length of instars leads the writer to believe that a greater or a less number is not only possible but probable.

Only a portion of the process of molting has been observed—the completion of a transformation from pupa to adult. The pupa had assumed a position head downward on the underside of a leaf which was inclined at an angle of about 15°. The legs were extended well apart, and the old exuvium was holding securely. When discovered, the adult was about half disclosed, escaping through a longitudinal slit in the dorsal median line of the pupal skin. It wriggled out until only the tips of the wings and of the third pair of legs were holding, then rested two or three minutes. When activity was resumed, the legs were repeatedly touched to the leaf. To obtain a better view, the writer then attempted to move the potted plant, upon which the insect hastily secured a footing on the leaf and turned around beside the cast skin, moving with quick nervous starts, and assumed an attitude of alert expectancy, waving its antennæ excitedly. The wings were crumpled and folded, but quickly assumed the shape and position normally found in the adult.

Adult coloration developed in less than two hours.

LENGTH OF ADULT LIFE

The males reared in 1914 were held without mating until death. They lived, respectively, 33, 8, 11, and 18 days. The female was mated, producing 8 eggs and living 6 days. This gives an average adult life of 15.2 days. In 1916 only one reared adult, a male, was confined until death. It was kept unmated and lived 39 days after maturity.

Thirteen females, collected in 1913 and confined with males for eggs, lived an average of 12 days, ranging from 9 to 19. Ten collected males that were mated gave an average also of 12, varying from 9 to 18.

LENGTH OF LIFE CYCLE

At Garden City, during 1914, the average temperature being 79.78° F., the different stages from deposition of the egg to death of the resulting individual were determined as follows:

	Days.
Egg stage	4
Nymphal stage	20.35
Maturity to mating	3
Mating to oviposition	1
Beginning oviposition to death	12
Total	40.35

The period from beginning of oviposition to death, to which reference is made on page 575 under "Length of adult life," is the average of the 23 collected individuals. To give an average for the period, an insufficient number of specimens were reared and kept under observation after being mated.

REPRODUCTIVE HABITS

Newly matured females have never been observed to mate in less than three days. Eggs are not secured until one day later. Subsequent mating is frequent and promiscuous, though a female often rejects a male.

A study was made of the reproductive activities for one day, of the rate of oviposition at various hours of the day, and of the seasonal variation in oviposition.

Five pairs of *Nysius ericae* were collected and each pair was confined in a cotton-stoppered vial. They were watched continuously for eight hours, remaining in the vials during the succeeding night. Table V is a typical record. Observations began at 7.45 a. m.[1]

TABLE V.—*Twenty-four-hour record of the reproductive activities of Nysius ericae, pair 3*

Paired 9.43.	Paired 11.57.	Paired 1.15.
Parted 9.46.	Parted 11.59½.	Parted 1.17½.
Paired 10.21.	Oviposited 12.45 to 12.59 (4 eggs).	Paired 1.27.
Parted 10.25.	Paired 1.02.	Parted 1.29.
Paired 10.56½.	Parted 1.07.	Paired 1.33.
Parted 11.01.	Paired 1.09½.	Parted 1.35.
Oviposited 11.22 to 11.24½ (8 eggs).	Parted 1.10.	Paired 3.35.
		Parted 3.37.

When examined the next morning at 7.45, both insects were living, but no more eggs had been deposited.

To ascertain the rate of oviposition at various hours of the day, several pairs were confined and the eggs removed daily at 8 a. m., 1 p. m., and 5 p. m. The experiment continued from June 6 to August 5, the pairs being replaced as they died, and by the latter date a total of 463 eggs had been secured. Of this number 239 were secured at 8 a. m., 145 at 1 p. m., and 79 at 5 p. m., or in about the ratio of 3 : 2 : 1. As nearly as can be determined, feeding and reproductive activity cease during darkness. This indicates that as many eggs are deposited during the late evening and early morning as are deposited from 8 a. m. to 5 p. m., and that twice as many eggs are deposited from 8 a. m. to 1 p. m. as from 1 p. m. to 5 p. m.

This decrease in reproductive activity during the afternoon has been observed under field conditions, the insects feeding less and seeking shade through the hot part of the afternoon.

[1] References to clock time refer to standard time.

To secure data on the seasonal variation in oviposition, females were collected and confined with males, and a record kept of the egg production and of the length of life of each female. The experiment was begun on May 6 and continued until September 18. Fifty-four females collected during May deposited 260 eggs in 295 days of life; 48 females collected during June deposited 231 eggs in 166 days of life; 39 females collected during July deposited 28 eggs in 216 days of life; 10 females collected during August deposited 17 eggs in 55 days of life, and 21 females collected during September deposited 113 eggs in 119 days of life. This is at the rate during May of 1 egg per female in 1.13 days; during June, 1 egg per female in 0.718 days; during July, 1 egg per female in 7.86 days; during August, 1 egg per female in 3.235 days; and during September, 1 egg per female in 1.05 days.

These figures coincide with the reproductive activity of the species as observed in the field. During May and June and again during September and October the females mate and oviposit frequently. Beginning in July and continuing into August they are much less prolific, it being sometimes difficult to secure sufficient eggs for rearing experiments. This decreased activity is exhibited only by the adults, there being no lengthening of the incubation period or nymphal instars.

EFFECT OF TEMPERATURE ON DEVELOPMENT

With an average temperature of 79.78° F. at Garden City, Kans., eggs hatched in about 4 days, and the average nymphal period (calculated from the averages of the largest number available in each instar) is 20.35 days. The average time, therefore, from oviposition to maturity is 24.35 days.

With an average temperature of 74.75° at Wichita, eggs hatched in 3.5 days, and the average nymphal period was 20.69 days. This gives the time from oviposition to maturity as 24.19 days.

Of 6 eggs deposited October 16 and 17, 1914, two hatched November 25, one November 28, and one November 30, making the shortest possible incubation period 39 days and the longest 44 days. Twenty-six times during this period the temperature was below 32° F., the minimum being 18° and the average 49.8°. This shows an average difference of 1.16 days in the length of the incubation period for each degree of difference in average temperatures.

From the data in the preceding paragraphs it appears that development at a temperature of 74.75° requires only 0.34 of a day longer than development at a temperature of 79.78°, but at 79.78° incubation requires only 4 days, while at a temperature of 49.8° 39 days are required, or $1\frac{1}{6}$ days for each degree of difference in temperature.

Of the 6 eggs under observation at a temperature of 18° four survived, and it is not certain that the other two were killed by the cold.

NUMBER OF GENERATIONS ANNUALLY

From the data on "Length of life cycle," p. 575, the time from oviposition by one generation to oviposition by the next succeeding generation is found to be 28.35 days, the temperature being 79.78° F. In some individuals this time is shortened in the nymphal period alone by at least two days.

These figures indicate that during the period in which they were collected a generation might become mature in less than one month. During the preceding April and May the temperatures averaged respectively 53.48° and 61.0°, and during the succeeding October 58.27°. These temperatures were above the average of 49.8° at which incubation proceeded late in October and during November of the same year.

Observations of 1913 showed that five generations matured after June 1. Hibernating nymphs from 1913 or overwintered eggs that hatched early in 1914 gave rise to a generation that completed development during the last two weeks of April and oviposited early in May. As the species was not reared continuously throughout the year the number of generations annually must be deduced from the data given.

The writer regards it as conclusive that five generations of the false chinch bug matured at Garden City, Kans., during 1913 after June 1. It is safe to regard this number as the minimum for seasons of the same length. The species hibernates either as an egg or as a young nymph which completes development very early the next spring. At Garden City the overwintering forms matured during April and deposited their eggs early in May. In seasons having temperatures above the average at Garden City the generation hatching from these eggs would become nearly mature by June.

To summarize: To the minimum of five generations, an overwintering generation and a possible generation in the spring may be added, making seven in all. In seasons in which the average temperature falls below that under which these studies were pursued the number of generations will be less, and with higher average temperatures may become greater.

PLATE 60

Nysius ericae: Adult. ×24

A
B
E
C
D

PLATE 61

Nysius ericae: Nymphal instars, greatly enlarged

A.—First-instar nymph.
B.—Second-instar nymph.
C.—Third-instar nymph.
D.—Fourth-instar nymph.
E.—Fifth-instar nymph, or pupa.

COMPARATIVE TRANSPIRATION OF CORN AND THE SORGHUMS

By EDWIN C. MILLER, *Associate Plant Physiologist, Department of Botany, Kansas Agricultural Experiment Station*, and W. B. COFFMAN, *Graduate Student, Kansas Agricultural College*

INTRODUCTION

In connection with the investigations of the water relations of corn. (*Zea mays*) and the sorghums (*Andropogon sorghum*) previously reported by the senior writer,[1] it was thought advisable to study the daily transpiration of these plants. A knowledge of the rate of transpiration of these plants under the same environment is essential in order to determine the factors that are concerned in their relative ability to withstand severe climatic conditions. A study of the rate of transpiration at various stages of the development of these plants should help to determine whether the ability of the sorghums to withstand severe climatic conditions better than the corn plant is aided by the power of the sorghums to retard the rate of water loss from their leaves or by the fact that they have a much smaller leaf surface exposed for the evaporation of water The experiments herein reported were conducted during the summers of 1916 and 1917 at the State Branch Experiment Station at Garden City, Kans.

EXPERIMENTAL METHODS

CULTURAL METHODS

The plants used in these experiments in 1916 were Pride of Saline corn, Dwarf Blackhull kafir, Dwarf milo, and Blackhull kafir. In 1917 in addition to these, Sherrod's White Dent corn, Freed's White Dent corn, Red Amber sorgo, Freed's sorgo, and feterita were used. These plants were grown in large galvanized-iron cans. The cans were 24 inches in height, with a diameter of 15 inches, and under the conditions of these experiments contained about 120 kilos of soil. The soil was worked through a ¼-inch mesh screen and was thoroughly tamped in the cans. The soil used in 1916 had a moisture content of 18 per cent and a wilting coefficient of 11.1, while the moisture content of the soil used in 1917 was 22 per cent and had a wilting coefficient of 15.1. Thus, for both seasons there was a difference of approximately 7 per cent between the water

[1] MILLER, E. C. COMPARATIVE STUDY OF THE ROOT SYSTEMS AND LEAF AREAS OF CORN AND THE SORGHUMS. *In* Jour. Agr. Research, v. 6, no. 9, p. 311–332, 3 fig. pl. 38–44. 1916. Literature cited, p. 331.
——— RELATIVE WATER REQUIREMENT OF CORN AND THE SORGHUMS. *In* Jour. Agr. Research, v. 6, no. 13, p. 473–484, 1 fig., pl. 70–72. 1916.
——— DAILY VARIATION OF WATER AND DRY MATTER IN THE LEAVES OF CORN AND THE SORGHUMS. *In* Jour. Agr. Research, v. 10, no. 1, p. 11–45, 10 fig., pl. 3. 1917.

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content of the soil used and its wilting coefficient. The method of sealing the cans and watering the plants was the same as that used in the determination of the water requirement of corn and the sorghums.[1] During the period of the experiment water was added to the cans from three to four times each day, in order to keep the moisture content of the soil as nearly constant as possible.

The number of plants was reduced to one to each can for Blackhull kafir, Red Amber sorgo, and the different varieties of corn, while for Dwarf milo, feterita, Dwarf Blackhull kafir, and Freed's sorgo the number of plants varied from one to three to each can. The number of plants in each is shown in the tables that record the data of the different experiments. The plants thus grown were as large and vigorous as those growing in the field under favorable conditions (Pl. 62–63). All the leaves remained vigorous long after the plants had reached their full vegetative growth, while all the plants that were allowed to mature produced a normal yield of grain.

EVAPORATION

The evaporation was determined by means of the Livingston porous-cup atmometers, and the cups used both in 1916 and 1917 had a coefficient of 74. These atmometers were connected with burettes that were graduated to 0. 1 c. c., and readings were made every two hours at the time of weighing the cans. In 1916 the atmometers were placed at a height of 2 feet in the open, while in 1917 they were placed at the same height in the center of a plot that was planted to corn. The cups were thus shaded after the corn had reached a height of 2 feet, so that the rate of evaporation as shown in the results was not so high in 1917 as in 1916.

DETERMINATION OF TRANSPIRATION

The cans were mounted on small wooden platforms provided with castors and were moved about on tracks made of unmatched boards. The cans were left in the open on the surface of the ground for the experiments in 1916, but in 1917 they were placed in a pit in the center of a plot that was planted to corn. The pit was of such a depth that the surface of the cans were on a level with the top of the ground (Pl. 62, C). This arrangement placed the plants more nearly under field conditions and reduced to a minimum the injury to the leaves on account of the prevailing high winds.

The plants were weighed on scales of the platform type that had a carrying capacity of 180 kilos and were sensitive to about 5 gm. The cans were weighed in a scale house in order to avoid any error in weight caused by the wind. The time required to bring the three or four plants used in the experiment to the scale house, weight them, and return them

[1] Miller, E. C. op. cit.

to their position in the open amounted to five or six minutes. The loss of water from the plants was determined in most of the experiments every two hours from 7 a. m. to 7 p. m. Determinations were made in two experiments in 1916 for each 2-hour period of the night, but since the loss of water from the plants during the night was very small, it was not deemed of sufficient importance to carry the night experiments further.

After an experiment had been completed the leaves and sheaths of the plants were cut in convenient lengths and the outline of these portions carefully traced on unruled paper with a hard lead pencil. The areas inclosed by these outlines were then determined by means of a polar planimeter. From the areas thus obtained the rate of transpiration per unit of leaf surface was calculated.

EXPERIMENTAL DATA

Five experiments were conducted in 1916 and eight in 1917. In the former year three varieties of plants were used in each experiment, while in 1917 four varieties were used. With the exception of two experiments in 1916, the transpiration was determined every two hours from 7 a. m. to 7 p. m. and for a period of two or three days. The results of the different experiments are shown in Tables II and III. The loss of water is expressed in grams per plant per hour, while the rate of transpiration is expressed in grams per square meter of leaf surface per hour and in grams per square meter of combined leaf and sheath surface per hour. The average hourly evaporation for each 2-hour period is also recorded. The data shown in these tables are expressed graphically in figures 1 to 13. In these figures the relative leaf surface of the plants used in each experiment is also shown. A general description of the plants used in each experiment is given in Table I.

TABLE I.—*General description of the plants used in transpiration experiments during the summers of 1916 and 1917 at Garden City, Kans.*

Date.	Plant.	Number of plants.	Height of plants.		Leaf surface per plant.	Sheath surface per plant.	General remarks.[a]
			Ft.	*in.*	*Sq. cm.*	*Sq. cm.*	
1916.							
July 6–9	Corn, Pride of Saline	1	2	8	10,481	419	9F and 3P unfolded leaves.
	Kafir, Dwarf Blackhull	1	2	0	5,179	219	8F and 2P unfolded leaves.
	Milo, Dwarf	2	2	4	3,225	403	9 leaves. Booting.
July 10–13	Corn, Pride of Saline	1	2	10	10,701	512	9F and 4P unfolded leaves.
	Kafir, Blackhull	1	2	1	6,069	272	8F and 3P unfolded leaves.
	Milo, Dwarf	2	2	7	3,134	541	9 leaves. Booting.
July 17–18	Corn, Pride of Saline	1	4	0	13,119	836	11F and 4P unfolded leaves.
	Kafir, Dwarf Blackhull	1	2	6	6,527	448	12 leaves. Booting.
	Milo, Dwarf	2	4	6	2,728	422	9 leaves. Blooming.
July 26–27	Corn, Pride of Saline	1	5	0	13,029	1,124	14 leaves. Tasseling.
	Kafir, Blackhull	1	3	0	10,101	566	11F and 2P unfolded leaves.
	Milo, Dwarf	2	4	6	2,825	387	9 leaves. Grain in the milk.
July 31–Aug. 2	Corn Pride of Saline	1	6	0	10,668	1,070	15 leaves. Ears forming.
	Kafir, Dwarf Blackhull	3	4	0	6,186	381	11 leaves. Blooming.
	Milo, Dwarf	3	4	6	2,580	390	9 leaves. Seed in dough stage.
1917.							
July 10–12	Corn, Freed's White Dent.	1	2	0	8,126	319	9 F and 4P unfolded leaves.
	Feterita	2	2	0	3,944	407	11 leaves. Booting.
	Sorgo, Freed's	2	2	0	3,236	242	8F and 2P unfolded leaves.
	Milo, Dwarf	2	1	4	5,780	207	10 F and 3P unfolded leaves.
July 13–16	Corn, Pride of Saline	1	3	4	9,664	505	8F and 4P unfolded leaves.
	Corn, Sherrod's White Dent.	1	3	4	6,264	545	8F and 4P unfolded leaves.
	Sorgo, Freed's	3	3	6	3,154	442	10 leaves. Booting.
	Kafir, Dwarf Blackhull	2	1	4	3,965	286	8F and 3P unfolded leaves.
July 17–19	Corn, Freed's White Dent.	1	3	9	11,225	662	10F and 5P unfolded leaves.
	Feterita	2	3	9	2,953	335	10 leaves. Heading.
	Kafir, Dwarf Blackhull	2	2	9	5,255	234	9F and 2P unfolded leaves.
	Milo, Dwarf	2	2	0	6,398	333	10 leaves. Booting.
July 20–22	Corn, Pride of Saline	1	3	4	12,989	518	10 F and 4P unfolded leaves.
	Sorgo, Freed's	3	5	10	3,029	330	10 leaves. Blooming.
	Sorgo, Red Amber	1	4	0	4,001	686	10 leaves. Booting.
	Milo, Dwarf	2	3	0	7,464	437	12 leaves. Booting.
July 23–25	Corn, Sherrod's White Dent.	1	4	0	10,916	840	16 leaves. Shooting.
	Feterita	1	5	0	3,624	408	12 leaves. Blooming.
	Sorgo, Freed's	2	6	3	2,951	380	9 leaves. Blooming.
	Milo, Dwarf	2	3	3	6,330	513	11 leaves. Heading.
July 26–27	Corn, Pride of Saline	1	3	8	16,943	798	12 F and 4P unfolded leaves.
	Sorgo, Freed's	2	6	3	2,793	585	9 leaves. Blooming
	Sorgo, Red Amber	1	3	8	4,843	634	12 leaves. Heading.
	Kafir, Dwarf	2	2	6	6,710	422	11 leaves. Booting.
July 30–31	Corn, Freed's White Dent.	1	4	4	15,205	997	14F and 3P unfolded leaves.
	Feterita	2	5	0	3,601	421	10 leaves. Finished blooming.
	Sorgo, Red Amber	1	5	6	3,973	660.	10 leaves. Blooming.
	Kafir, Dwarf Blackhull	2	3	0	7,159	430	14 leaves. Booting.
August 2–3	Corn, Freed's White Dent	1	6	0	14,568	1,334	16 leaves. Shooting.
	Corn, Sherrod's White Dent.	1	5	6	10,825	960	12 leaves. Grain in milk
	Feterita	2	4	9	3,038	333	8 leaves. Seed forming.
	Sorgo, Red Amber	1	6	0	4,131	646	10 leaves. Seed forming.

[a] F=Leaves fully unfolded: P=leaves partially unfolded.

TABLE II.—*Rate of the hourly transpiration of corn and the sorgums at Garden City, Kans., in 1916*[a]

Period ending—	Evaporation per hour.	CORN, PRIDE OF SALINE (1).			KAFIR, DWARF BLACKHULL (1).			MILO, DWARF (2).		
		Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.
	C. c.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
July 5:										
3 p. m.	6.4	185	175.8	169.2	57	109.1	104.7	62	191.4	167.9
5 p. m.	5.1	117	111.5	107.3	39	75.3	72.3	43	131.7	115.6
July 6:										
7 a. m.	1.1	13	12.9	12.4	4	8.1	7.8	3	10.5	9.2
9 a. m.	2.0	77	73.9	71.1	21	40.1	38.9	28	87.6	76.8
11 a. m.	3.2	107	102.4	97.7	49	95.6	91.7	44	137.2	120.3
1 p. m.	4.7	155	148.2	142.6	89	170.9	164.0	75	230.9	202.6
3 p. m.	5.5	184	175.4	168.7	81	157.4	151.0	58	180.6	158.4
5 p. m.	5.3	124	118.2	113.7	43	82.1	78.7	46	142.6	125.1
7 p. m.	3.9	63	60.5	58.2	11	20.3	19.5	11	38.3	29.2
July 7:										
7 a. m.	.8	8	7.9	7.7	4	7.9	7.6	3	8.3	7.3
9 a. m.	4.7	135	128.2	123.3	57	110.1	105.6	51	158.9	139.4
11 a. m.	6.4	205	195.4	188.0	85	163.2	156.6	87	268.1	235.2
1 p. m.	8.1	176	167.7	161.4	92	177.7	170.5	83	257.0	225.7
3 p. m.	8.6	205	195.8	188.4	103	197.9	189.9	79	246.5	216.2
5 p. m.	8.4	187	178.7	171.9	81	157.4	151.0	71	219.3	192.4
7 p. m.	5.0	74	70.5	67.9	7	13.5	13.0	18	55.0	48.3
July 8:										
7 a. m.	.8	5	4.8	4.6	5	9.2	8.8	2	7.4	6.5
9 a. m.	4.6	135	128.2	123.3	57	109.1	104.7	62	191.4	167.9
11 a. m.	6.1	213	202.5	194.9	85	164.1	157.5	79	246.5	216.2
1 p. m.	7.1	233	222.5	214.5	123	238.5	228.8	94	290.6	245.9
3 p. m.	8.1	219	208.7	200.8	110	212.4	203.8	108	334.8	293.7
5 p. m.	7.9	201	192.0	184.8	85	164.1	157.5	64	197.6	173.3
7 p. m.	6.7	109	104.4	100.4	25	47.3	45.4	25	76.7	67.3
July 9:										
7 a. m.	1.0	12	11.8	11.4	7	12.6	12.0	4	13.2	11.2
11 a. m.	6.6	195	185.4	178.4	97	187.8	180.2	90	287.9	245.5
3 p. m.	9.3	265	252.8	243.2	141	273.2	262.2	127	403.5	344.1
7 p. m.	8.3	156	148.3	142.7	60	155.4	149.2	52	166.7	142.1
July 10:										
7 a. m.	1.6	15	14.1	13.5	4	7.9	7.6	4	13.2	11.2
9 a. m.	5.2	155	145.4	138.3	103	169.7	162.4	64	203.4	173.4
11 a. m.	8.1	241	224.8	214.6	117	191.9	183.7	117	372.4	317.6
1 p. m.	10.1	293	274.4	261.8	131	215.8	206.6	138	439.4	374.7
3 p. m.	10.8	290	271.1	258.7	159	262.8	251.5	129	412.3	351.6
5 p. m.	9.9	230	215.0	205.2	120	197.7	189.2	103	327.0	278.8
7 p. m.	7.5	109	102.4	97.7	32	52.7	50.5	35	113.2	96.6
July 11:										
7 a. m.	1.5	9	8.8	8.4	5	8.8	8.4	4	13.2	11.2
9 a. m.	4.5	106	99.1	94.6	57	93.1	89.1	39	124.4	106.1
11 a. m.	6.3	145	135.6	129.4	74	121.9	116.7	71	225.7	192.4
1 p. m.	7.3	247	231.4	220.8	106	174.6	167.1	104	332.6	283.6
3 p. m.	7.4	233	218.3	208.3	127	210.0	201.0	104	332.6	283.6
5 p. m.	6.1	187	175.3	167.3	92	151.6	145.1	71	225.7	192.4
July 12:										
8 a. m.	.6	14	12.8	12.2	8	13.2	12.6	5	16.1	13.7
9 a. m.	3.7	170	158.9	151.7	35	57.7	55.2	35	113.2	96.6
11 a. m.	5.1	209	194.9	186.0	99	163.1	156.1	94	299.1	255.0
1 p. m.	6.4	219	205.2	195.8	103	169.7	162.4	95	304.6	259.8
3 p. m.	4.1	106	99.1	94.6	103	183.3	174.3	60	192.2	163.9
5 p. m.	3.5	65	63.7	57.7	43	76.0	72.3	27	84.5	72.1
7 p. m.	2.7	21	20.1	19.2	14	25.0	23.8	27	84.5	72.1
July 13:										
7 a. m.	.7	8	7.7	7.4	7	11.6	11.1	3	10.4	8.8
9 a. m.	3.7	131	141.1	133.8	75	133.2	126.7	48	152.3	129.9
11 a. m.	5.6	191	205.8	195.0	103	183.3	174.3	85	271.1	231.2
1 p. m.	5.9	212	228.4	216.5	106	189.5	180.2	97	310.2	264.5
3 p. m.	7.1	273	293.6	278.2	152	271.8	258.4	121	384.4	327.8
July 17:										
9 a. m.	5.8	213	162.0	152.3	95	146.1	136.9	73	265.3	230.0
11 a. m.	8.4	241	183.3	172.4	109	167.5	156.9	92	336.7	291.9
1 p. m.	9.3	237	180.7	169.8	159	243.3	227.8	108	395.3	342.7
3 p. m.	9.6	265	202.4	190.3	145	221.9	207.8	119	433.7	376.0
5 p. m.	8.9	255	194.4	182.7	121	184.4	172.7	94	343.1	297.5
7 p. m.	6.1	109	83.5	78.5	57	86.4	81.0	37	136.3	118.2

[a] The figures inclosed in parentheses represent the number of plants to each can.

TABLE II.—*Rate of the hourly transpiration of corn and the sorghums at Garden City, Kans., in 1916*—Continued

Period ending—	Evaporation per hour.	CORN, PRIDE OF SALINE (1).			KAFIR, DWARF BLACKHULL (1).			MILO, DWARF (2).		
		Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.
	C. c.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
July 18:										
7 a. m.	1.5	23	17.5	16.5	5	7.3	6.8	9	31.1	27.0
9 a. m.	6.1	191	145.6	136.9	113	172.9	161.9	57	206.8	179.3
11 a. m.	9.0	244	186.0	174.9	135	205.8	192.7	85	310.2	268.9
1 p. m.	8.8	244	186.0	174.9	138	211.1	197.8	89	323.9	280.8
3 p. m.	8.4	255	194.0	182.4	163	249.4	233.6	106	388.0	336.3
5 p. m.	8.2	199	151.3	142.3	77	118.6	111.1	60	220.5	191.2
July 26:										
8 a. m.	3.9	121	92.5	85.1	75	73.7	69.5	35	125.5	110.4
10 a. m.	6.7	212	162.7	149.8	149	151.5	143.2	79	281.1	247.3
12 noon	8.2	291	223.0	205.3	177	180.5	170.7	99	350.1	307.9
2 p. m.	9.5	329	252.5	232.5	219	223.9	211.6	122	432.4	379.5
4 p. m.	9.5	259	198.4	182.7	213	216.7	204.9	117	412.8	363.1
6 p. m.	9.1	236	181.1	166.8	127	130.1	122.9	58	206.0	181.2
8 p. m.	6.7	164	125.9	115.9	31	34.8	32.7	27	93.7	82.4
10 p. m.	3.9	11	8.1	7.4	18	19.9	18.7	3	12.4	10.9
July 27:										
12 midnight	3.4	25	19.2	17.7	14	15.4	14.5	13	44.2	38.9
2 a. m.	1.6	25	19.2	17.7	3	4.0	3.6	5	18.6	16.3
4 a. m.	.8	18	13.8	12.7	3	4.0	3.6	2	6.2	5.4
6 a. m.	.9	31	24.2	22.3	15	16.0	15.1	5	18.6	16.3
8 a. m.	2.7	71	54.5	50.2	57	62.3	58.7	21	75.1	66.1
10 a. m.	4.9	149	114.0	104.9	92	101.5	95.5	57	206.7	176.5
July 31:										
10 a. m.	4.9	191	179.0	162.7	75	121.8	114.7	58	224.3	194.8
12 noon	6.5	237	222.1	201.9	104	167.8	158.1	75	292.8	254.3
2 p. m.	6.9	255	238.5	216.8	112	181.0	170.5	83	319.9	277.9
4 p. m.	7.3	241	225.4	204.8	94	152.5	143.6	70	270.2	234.7
6 p. m.	6.1	167	156.0	141.8	49	80.3	75.6	37	142.2	123.5
8 p. m.	2.6	29	26.7	24.3	3	5.7	5.3	8	31.7	27.5
August 1:										
8 a. m.	1.5	22	20.8	18.9	7	11.3	10.7	5	18.1	15.7
10 a. m.	6.0	202	189.3	172.0	71	114.5	107.9	57	219.1	190.3
12 noon	7.9	276	258.7	235.1	112	181.0	170.5	85	329.6	286.3
2 p. m.	9.1	305	285.8	259.3	119	192.4	181.2	97	376.1	326.2
4 p. m.	9.1	315	294.7	267.9	121	196.4	185.0	95	370.3	321.7
6 p. m.	8.1	241	225.4	204.8	89	143.1	134.8	71	274.7	238.6
8 p. m.	6.8	81	76.4	69.4	14	22.9	21.6	21	82.1	71.3
10 p. m.	4.3	31	29.5	26.8	5	7.5	7.1	2	9.0	7.9
August 2:										
12 midnight	3.3	18	16.9	15.3	3	5.7	5.3	4	14.2	12.4
2 a. m.	2.6	35	32.8	29.8	4	5.9	5.6	2	9.0	7.9
4 a. m.	2.1	15	13.6	12.3	5	7.5	7.1	7	27.1	23.6
6 a. m.	1.6	21	17.5	15.9	12	14.5	13.7	8	28.7	24.9
8 a. m.	4.4	135	126.1	114.6	41	66.5	62.7	34	132.5	115.1
10 a. m.	7.5	227	212.3	192.9	89	143.1	134.8	75	292.8	254.3
12 noon	9.7	290	271.8	246.9	114	184.8	174.1	88	343.2	298.1

TABLE III.—*Rate of hourly transpiration of corn and the sorghums at Garden City, Kans., in 1917* [a]

Period ending—	Evaporation per hour.	MILO, DWARF (2).			SORGO, FREED'S (2).			FETERITA (2).			CORN, FREED'S WHITE DENT (1).		
		Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.
July 10:	*C.c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
9 a. m.	1.8	50	86.6	83.6	34	105.1	97.7	43	109.0	98.9	85	104.5	100.7
11 a. m.	3.4	71	122.8	118.6	55	170.0	158.0	66	167.3	151.7	89	109.5	105.5
1 p. m.	4.5	87	150.6	145.4	53	163.8	152.3	78	197.7	179.3	135	166.1	160.0
3 p. m.	5.1	87	150.6	145.4	55	170.0	158.0	80	202.8	183.9	142	174.7	168.2
5 p. m.	4.5	74	128.0	123.6	46	143.7	132.2	62	157.2	142.5	128	157.4	151.7
7 p. m.	3.9	28	48.4	46.8	16	49.5	46.0	23	58.3	52.9	50	61.5	59.2
July 11:													
7 a. m.	.8	3	5.2	5.0	3	9.3	8.6	4	10.1	9.2	6	7.4	7.1
9 a. m.	4.6	60	103.8	100.2	35	108.2	100.6	50	126.7	114.9	85	104.5	100.7
11 a. m.	7.9	84	145.4	140.4	57	176.2	163.8	74	187.5	170.1	174	214.0	206.2
1 p. m.	7.8	106	183.4	177.2	74	228.7	212.6	94	238.3	216.1	170	209.1	201.4
3 p. m.	8.0	101	174.8	168.8	64	197.8	183.9	101	256.0	232.2	184	226.3	218.0
5 p. m.	8.0	90	155.8	150.4	60	185.5	172.4	83	210.4	190.8	156	191.9	184.8
7 p. m.	5.6	35	60.6	58.4	21	64.9	60.3	34	86.2	78.2	78	95.9	92.4
July 12:													
7 a. m.	.8	1	1.8	1.6	2	6.2	5.7	4	10.1	9.2	7	8.6	8.3
9 a. m.	3.8	73	126.2	122.0	41	126.7	117.8	44	111.5	101.1	78	95.9	92.4
11 a. m.	5.5	101	174.8	168.8	64	197.8	183.9	85	215.6	195.4	159	195.6	188.4
1 p. m.	6.3	110	190.4	183.8	89	275.1	255.7	110	278.8	252.9	198	243.5	234.6
3 p. m.	7.7	122	211.0	203.8	97	299.8	278.7	115	291.5	264.4	230	282.9	272.5
5 p. m.	6.8	73	126.2	122.0	55	170.0	158.0	89	225.6	204.6	216	265.7	255.9
7 p. m.	5.5	37	64.0	61.8	35	108.2	100.6	51	129.3	117.2	99	121.8	117.3
		CORN, SHERROD'S WHITE DENT (1).			CORN, PRIDE OF SALINE (1).			KAFIR, DWARF BLACKHULL (2).			SORGO, FREED'S (3).		
July 13:													
9 a. m.	3.6	96	153.4	141.0	89	92.1	87.5	39	98.4	94.0	44	139.6	122.3
11 a. m.	7.9	135	215.7	198.2	167	172.9	164.2	60	151.3	144.6	91	288.6	253.0
1 p. m.	7.8	135	215.7	198.2	191	197.7	187.8	66	166.5	159.0	99	314.0	275.2
3 p. m.	6.3	145	231.6	212.9	163	168.7	160.3	71	179.1	171.1	99	314.0	275.2
5 p. m.	5.6	128	204.5	188.0	156	161.5	153.4	62	156.4	149.4	90	285.4	250 2
7 p. m.	4.7	71	113.4	104.3	92	95.2	90.5	27	68.1	65.1	44	139.6	122.3
July 14:													
7 a. m.	1.0	4	6.4	5.9	6	6.2	5.9	1	2.5	2.4	3	9.5	8.3
9 a. m.	5.1	71	113.4	104.3	89	92.1	87.5	39	98.4	94.0	37	117.3	102.9
11 a. m.	4.0	53	84.7	77.8	82	84.9	80.6	27	68.1	65.1	30	95.1	83.4
1 p. m.	3.5	53	84.7	77.8	71	73.5	69.8	35	88.3	84.3	34	107.8	94.5
3 p. m.	3.5	86	137.4	126.3	124	128.4	121.9	35	88.3	84.3	43	136.4	119.6
5 p. m.	3.8	86	137.4	126.3	99	102.5	97.3	43	108.5	103.6	45	142.7	125.1
7 p. m.	2.5	53	84.7	77.8	32	33.1	31.6	12	30.3	28.9	12	38.1	33.4
July 15:													
7 a. m.	.6	2	3.2	2.9	5	5.2	4.9	1	2.5	2.4	3	9.5	8.3
9 a. m.	3.6	60	95.8	88.1	92	95.2	90.5	48	121.1	115.7	40	126.8	111.2
11 a. m.	3.9	113	180.5	165.9	152	157.3	149.5	55	138.7	132.5	56	177.6	155.7
1 p. m.	4.7	117	186.9	171.8	156	161.5	153.4	62	156.4	149.4	70	222.0	194.6
3 p. m.	4.0	103	164.5	151.2	135	139.8	132.7	50	126.1	120.5	58	183.9	161.3
5 p. m.	3.2	92	147.0	135.1	99	102.5	97.3	35	88.3	84.3	43	136.4	119.6
7 p. m.	2.7	53	84.7	77.8	50	51.7	49.2	11	27.7	26.5	14	44.4	38.6

[a] The figures inclosed in parentheses represent the number of plants to each can.

TABLE III.—*Rate of hourly transpiration of corn and the sorghums at Garden City, Kans., in 1917*—Continued

Period ending—	Evaporation per hour.	MILO, DWARF (2).			KAFIR, DWARF BLACKHULL (2).			CORN, FREED'S WHITE DENT (1).			FETERITA (2).		
		Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.
July 17:	C.c.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
9 a. m.	3.3	57	89.1	84.6	34	64.7	61.9	117	104.3	98.4	32	108.3	97.2
11 a. m.	4.4	103	100.9	153.0	64	121.8	116.6	177	157.8	148.9	64	216.6	194.5
1 p. m.	6.1	120	187.5	178.3	83	157.9	151.2	206	183.6	173.3	97	328.2	294.8
3 p. m.	6.7	118	184.4	175.3	83	157.9	151.2	191	170.2	160.6	94	318.1	285.7
5 p. m.	5.9	83	129.7	123.3	64	121.8	116.6	152	135.4	127.8	60	203.0	182.4
7 p. m.	4.9	21	32.8	31.2	14	26.6	25.5	74	66.0	62.2	21	71.1	63.7
July 18:													
7 a. m.	.7	2	3.1	3.0	2	3.8	3.6	5	4.5	4.2	2	6.8	6.1
9 a. m.	2.8	74	115.6	110.0	67	127.5	122.0	128	114.1	107.7	41	138.7	124.6
11 a. m.	4.7	94	146.9	139.7	69	131.3	125.7	184	164.0	154.8	76	257.2	231.0
1 p. m.	6.4	122	190.0	181.3	106	201.7	193.1	241	214.8	202.7	110	372.3	334.3
3 p. m.	6.2	142	221.9	211.0	117	222.6	213.1	259	230.8	217.8	103	348.7	313.2
5 p. m.	4.6	74	115.6	110.0	62	118.0	112.9	188	167.6	158.1	62	209.8	188.4
7 p. m.	3.1	12	18.7	17.8	2	3.8	3.6	18	16.0	15.1	11	37.2	33.4
July 19:													
7 a. m.	.7	2	3.1	3.0	2	3.8	3.6	2	1.8	1.7	1	3.4	3.1
9 a. m.	2.6	60	93.7	89.2	43	81.9	78.3	135	120.3	113.5	37	125.2	112.5
11 a. m.	3.7	103	160.9	153.0	94	178.9	171.2	170	151.5	143.0	64	216.6	194.5
1 p. m.	4.8	126	196.9	187.2	97	184.6	176.7	213	189.8	179.1	96	324.9	291.6
3 p. m.	4.7	108	168.7	160.4	94	178.9	171.2	195	173.8	164.0	85	287.6	258.4
5 p. m.	4.7	94	146.9	139.7	67	127.5	122.0	156	139.0	131.2	66	223.2	200.4
7 p. m.	3.9	35	54.7	52.0	28	53.2	51.0	85	75.8	71.5	45	152.3	136.8
		MILO, DWARF (2).			SORGO, RED AMBER (1)			CORN, PRIDE OF SALINE (1).			SORGO, FREED'S (3).		
July 20:													
9 a. m.	3.0	101	135.2	127.8	67	167.5	142.9	124	95.5	91.8	40	151.8	136.0
11 a. m.	3.9	140	187.5	177.2	117	292.5	249.5	181	139.3	134.0	71	284.3	242.3
1 p. m.	4.9	170	227.7	215.2	138	345.0	294.2	241	185.6	178.4	103	339.0	300.5
3 p. m.	5.3	167	223.7	211.4	138	345.0	294.2	223	171.7	165.1	100	349.8	315.5
5 p. m.	4.9	135	180.8	170.9	103	257.5	219.6	206	158.6	152.4	95	313.5	282.7
7 p. m.	4.0	30	40.2	38.0	28	70.0	59.7	71	54.7	52.0	19	62.7	56.5
July 21:													
7 a. m.	.4	1	1.3	1.2	1	2.5	2.1	2	1.5	1.4	1	3.3	3.0
9 a. m.	2.9	53	71.0	67.1	32	94.1	78.2	67	51.5	49.0	33	108.9	98.2
11 a. m.	5.1	122	163.4	154.4	99	291.2	242.1	188	144.7	139.2	70	250.8	226.2
1 p. m.	7.8	167	223.7	211.4	149	438.2	364.3	246	189.4	182.1	100	349.8	315.5
3 p. m.	8.1	172	230.4	217.7	149	438.2	364.3	280	215.6	207.3	120	390.0	351.2
5 p. m.	6.5	106	142.0	134.2	74	217.7	180.9	145	111.0	107.3	65	214.5	193.5
7 p. m.	4.7	39	52.2	48.4	35	102.9	85.6	106	81.6	78.5	27	89.1	80.4
July 22:													
7 a. m.	1.7	6	8.0	7.6	4	11.8	9.8	15	11.5	11.1	5	16.5	14.9
9 a. m.	4.9	73	97.8	92.4	71	208.8	173.6	135	103.9	99.9	61	201.3	182.5
11 a. m.	5.7	126	168.8	159.5	71	208.8	173.6	174	133.9	128.8	65	214.5	193.5
1 p. m.	5.6	144	192.9	182.3	131	385.3	320.3	245	188.5	181.3	92	303.6	273.8
3 p. m.	5.9	170	227.7	215.2	135	397.0	330.1	237	182.4	175.4	97	320.1	288.7
5 p. m.	5.7	154	206.3	194.9	120	352.9	293.4	255	196.3	188.7	91	300.3	270.8
7 p. m.	4.6	60	80.4	76.0	46	135.3	112.7	135	103.9	99.9	30	99.0	89.3

TABLE III.—*Rate of hourly transpiration of corn and the sorghums at Garden City, Kans., in 1917*—Continued

Period ending—	Evaporation per hour.	MILO, DWARF (2).			SORGO, FREED'S (2).			FETERITA (1).			CORN, SHERROD'S WHITE DENT (1).		
		Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.
July 23:	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
9 a. m.	4.7	101	159.6	147.5	66	223.7	198.2	82	226.5	203.5	209	191.6	177.7
11 a. m.	6.1	145	229.1	211.8	106	359.3	318.3	103	284.5	255.6	298	273.1	253.4
1 p. m.	6.5	177	279.6	258.6	120	406.8	360.4	149	411.6	369.7	344	315.3	292.5
3 p. m.	7.3	183	289.1	267.3	119	403.4	357.4	135	372.9	335.0	312	286.0	265.3
5 p. m.	6.7	140	221.2	204.5	97	328.8	291.3	85	234.8	210.9	259	237.4	220.2
7 p. m.	5.1	46	72.7	67.2	37	125.4	111.1	46	127.1	114.1	103	94.4	87.6
July 24:													
7 a. m.	1.1	4	6.3	5.8	6	20.3	18.0	4	11.0	9.9	13	11.9	11.1
9 a. m.	3.5	78	123.2	114.0	58	196.6	174.2	60	165.7	148.9	195	178.7	165.8
11 a. m.	5.1	144	227.5	210.4	112	379.7	336.3	120	331.5	297.8	280	256.6	238.1
1 p. m.	6.7	181	285.9	264.4	124	420.3	372.4	152	419.9	377.2	330	302.5	280.6
3 p. m.	7.3	174	274.9	254.2	122	413.6	366.4	142	392.3	352.4	294	269.5	250.0
5 p. m.	6.3	131	207.0	191.4	85	288.1	255.3	99	273.5	245.7	234	214.5	198.8
7 p. m.	5.1	46	72.7	67.2	39	132.2	117.1	43	118.8	106.8	113	103.6	96.1
July 25:													
7 a. m.	.8	2	3.2	2.9	5	16.9	15.0	1	2.8	2.5	10	9.2	8.5
9 a. m.	2.3	60	94.8	87.7	37	125.4	111.1	53	146.4	131.5	142	130.1	120.7
11 a. m.	3.7	117	184.8	170.9	82	277.9	246.2	103	284.5	255.6	248	227.3	210.9
1 p. m.	[illegible].4	161	254.3	235.2	99	335.6	297.3	135	372.9	335.0	280	256.6	238.1
3 p. m.	4.9	154	243.3	225.0	103	349.2	309.3	124	342.5	307.7	266	243.8	226.2
5 p. m.	4.7	119	188.0	173.9	74	250.8	222.2	82	226.5	203.5	202	185.2	171.8
7 p. m.	3.2	43	67.9	62.8	35	118.6	105.1	39	107.7	96.8	99	90.7	84.2

Period ending—	Evaporation per hour.	SORGO, RED AMBER (1).			CORN, PRIDE OF SALINE (1).			KAFIR, DWARF BLACKHULL (2).			SORGO, FREED'S (2).		
July 26:													
9 a. m.	2.9	99	204.5	180.7	206	121.6	116.1	69	102.8	96.8	53	189.6	171.8
11 a. m.	4.9	142	293.4	259.1	280	165.3	157.8	110	163.9	154.3	85	304.1	275.5
1 p. m.	5.5	181	373.9	330.3	365	215.5	205.7	117	174.4	164.1	105	375.7	340.4
3 p. m.	5.6	206	483.6	421.3	337	198.9	190.0	126	187.8	176.7	106	379.2	343.6
5 p. m.	5.3	163	382.6	333.3	273	161.2	153.9	89	132.6	124.8	76	271.9	246.4
7 p. m.	4.1	53	124.4	108.4	159	93.9	89.6	39	58.1	54.7	41	146.7	132.9
July 27:													
7 a. m.	2.1	9	21.1	18.4	24	14.2	13.5	4	6.0	5.6	7	25.0	22.7
9 a. m.	4.0	96	225.4	196.3	198	116.9	111.7	82	122.2	115.0	62	221.8	201.0
11 a. m.	4.9	163	382.6	333.3	340	200.7	191.7	122	181.8	171.1	96	343.5	311.2
1 p. m.	6.7	202	474.2	413.1	376	222.0	212.0	140	208.6	196.4	126	450.8	408.4
3 p. m.	8.1	198	464.8	404.9	337	198.9	190.0	119	177.3	166.9	115	411.5	372.8
5 p. m.	7.3	142	293.4	259.1	291	171.8	164.1	97	144.6	136.0	78	279.0	252.8
7 p. m.	6.0	57	133.8	116.6	191	112.8	107.8	44	65.6	61.7	46	164.6	149.1

Period ending—	Evaporation per hour.	SORGO, RED AMBER (1).			KAFIR, DWARF BLACKHULL (2).			FETERITA (2).			CORN, FREED'S WHITE DENT (1).		
July 30:													
9 a. m.	3.7	71	178.8	153.3	76	106.1	100.1	67	186.1	166.7	159	177.3	161.9
11 a. m.	4.5	135	340.1	291.6	129	180.2	170.0	94	261.1	233.8	255	284.3	259.7
1 p. m.	6.7	198	498.7	427.6	173	241.6	227.9	142	394.4	353.2	305	340.0	310.6
3 p. m.	7.6	220	554.2	475.2	175	244.4	230.6	149	413.9	370.6	[a] 418	275.0	258.0
5 p. m.	6.8	138	347.6	298.1	128	178.8	168.7	87	241.7	216.4	315	207.2	194.4
7 p. m.	6.1	89	224.2	192.2	50	69.8	65.9	50	138.9	124.4	195	128.3	120.4
July 31:													
7 a. m.	2.7	13	32.7	28.1	7	9.8	9.2	7	19.4	17.4	31	20.4	19.1
9 a. m.	3.7	64	161.2	138.2	66	92.2	87.0	46	127.8	114.4	213	140.1	131.5
11 a. m.	5.2	128	322.4	276.4	160	223.5	210.8	103	286.1	256.2	308	202.6	190.1
1 p. m.	5.5	135	340.1	291.6	149	208.1	196.3	112	311.1	278.6	301	198.0	185.8
3 p. m.	9.0	163	410.6	352.1	161	224.9	212.2	115	319.4	286.0	354	232.9	218.5
5 p. m.	3.9	60	151.1	134.5	34	47.5	44.8	27	75.0	67.2	92	60.5	56.8
7 p. m.	2.7	28	70.5	60.5	12	16.8	15.8	12	33.3	29.9	67	44.1	41.4

[a] New plant substituted.

TABLE III.—*Rate of hourly transpiration of corn and the sorghums at Garden City, Kans., in 1917*—Continued

Period ending—	Evaporation per hour.	CORN, SHERROD'S WHITE DENT (1).			FETERITA (2).			SORGO, RED AMBER (1).			CORN, FREED'S WHITE DENT (1).		
		Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheaf surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.	Per plant.	Per square meter of leaf surface.	Per square meter of combined leaf and sheath surface.
August 2:	*C. c.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
9 a. m.	2.2	120	110.8	101.9	34	111.8	100.9	67	162.2	140.2	142	97.5	89.3
11 a. m.	3.4	234	216.1	198.6	64	210.5	189.9	113	273.6	236.4	280	192.2	176.1
1 p. m.	4.5	259	239.2	219.9	96	315.8	284.9	156	377.7	326.4	308	211.4	193.7
3 p. m.	5.1	291	268.7	247.0	99	325.7	293.8	184	445.5	384.9	330	226.5	207.5
5 p. m.	5.1	287	265.0	243.6	73	240.1	216.6	113	273.6	236.4	294	201.8	184.9
7 p. m.	3.0	113	104.3	95.9	27	88.8	80.1	46	111.3	96.2	131	89.9	82.4
August 3:													
7 a. m.	1.1	13	12.0	11.0	3	9.9	9.0	3	7.2	6.3	14	9.6	8.8
9 a. m.	3.1	181	167.1	153.7	35	115.1	103.9	60	145.3	125.5	191	131.1	120.1
11 a. m.	4.5	252	232.7	213.9	74	243.4	219.6	145	351.1	303.3	305	209.3	191.8
1 p. m.	4.7	287	265.0	243.6	106	348.7	314.5	177	428.6	370.3	319	218.9	200.6
3 p. m.	5.9	298	275.2	253.0	106	348.7	314.5	170	411.6	355.6	337	231.3	211.9
5 p. m.	5.5	266	245.6	225.8	76	250.0	225.5	145	351.1	303.3	294	201.8	184.9
7 p. m.	4.8	142	131.1	120.5	44	144.6	130.6	60	145.3	125.5	152	104.3	95.6

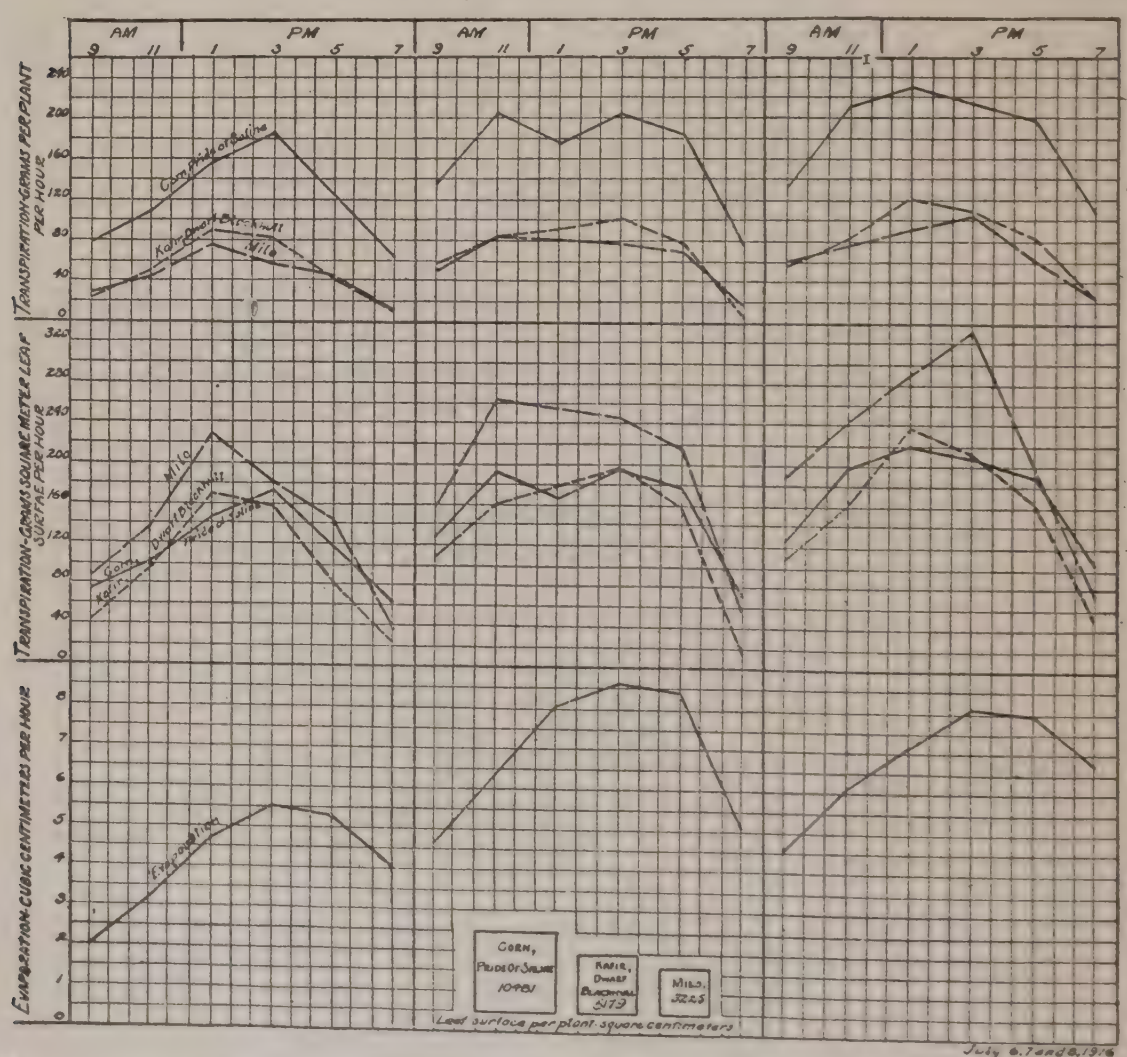

FIG. 1.—Graphs showing the amount of water transpired by Pride of Saline corn, Dwarf Blackhull kafir, and Dwarf milo during July 6, 7, and 8, 1916, and the evaporation during the corresponding period.

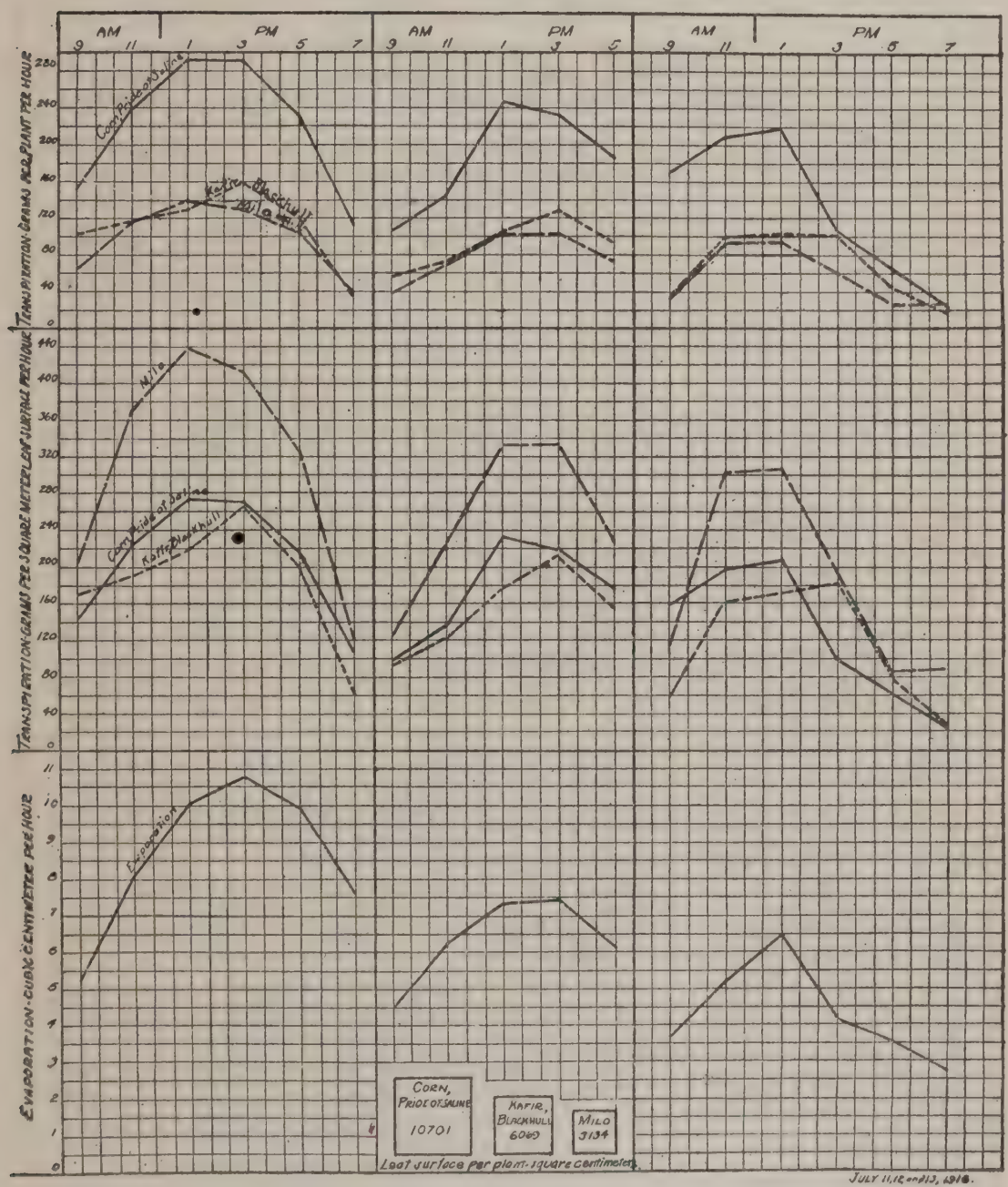

FIG. 2.—Graphs showing the amount of water transpired by Pride of Saline corn, Blackhull kafir, and Dwarf milo during July 11, 12, and 13, 1916, and the evaporation during the corresponding period.

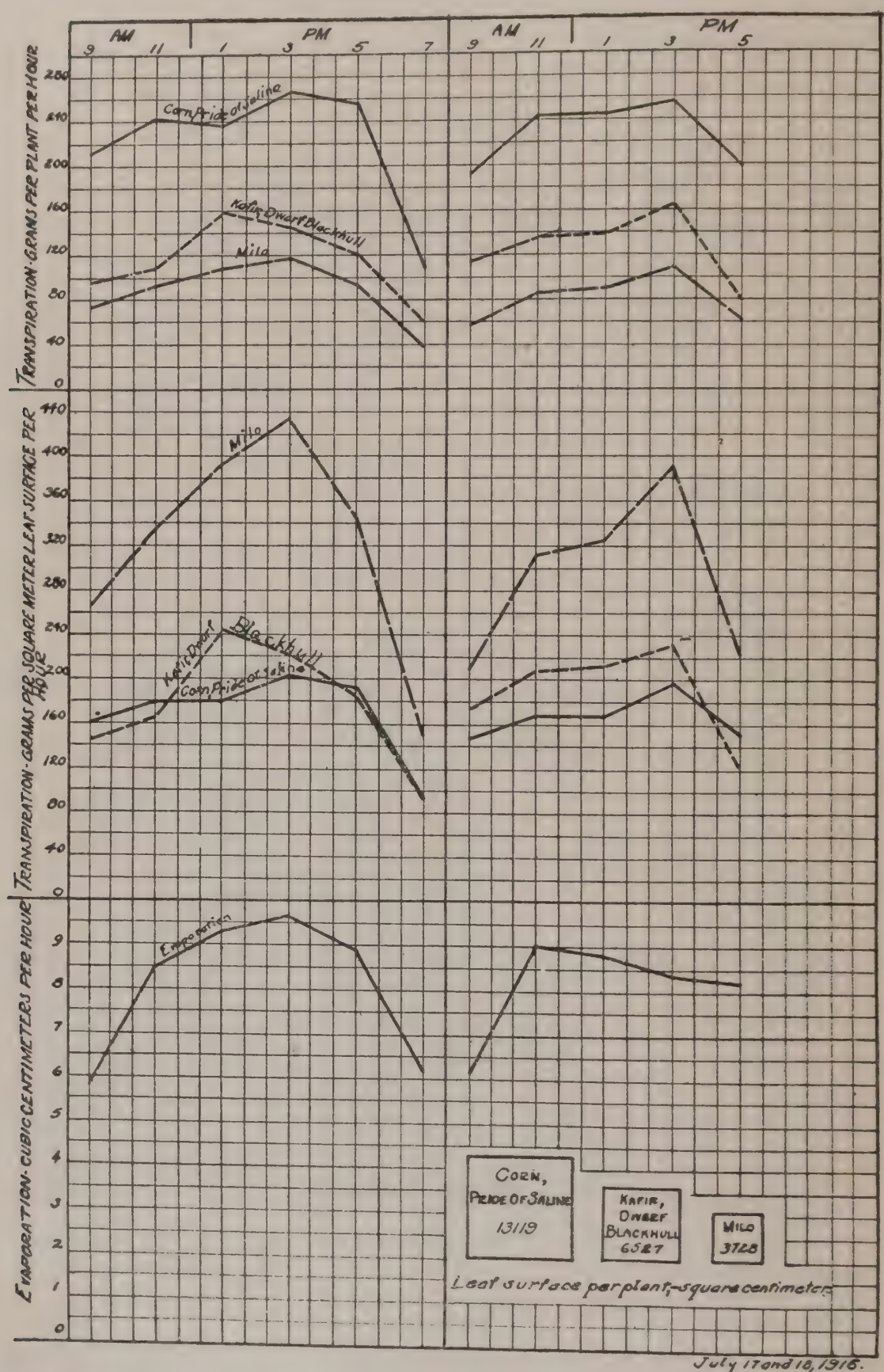

FIG. 3.—Graphs showing the amount of water transpired by Pride of Saline corn, Dwarf Blackhull kafir, and Dwarf milo during July 17 and 18, 1916, and the evaporation during the corresponding period.

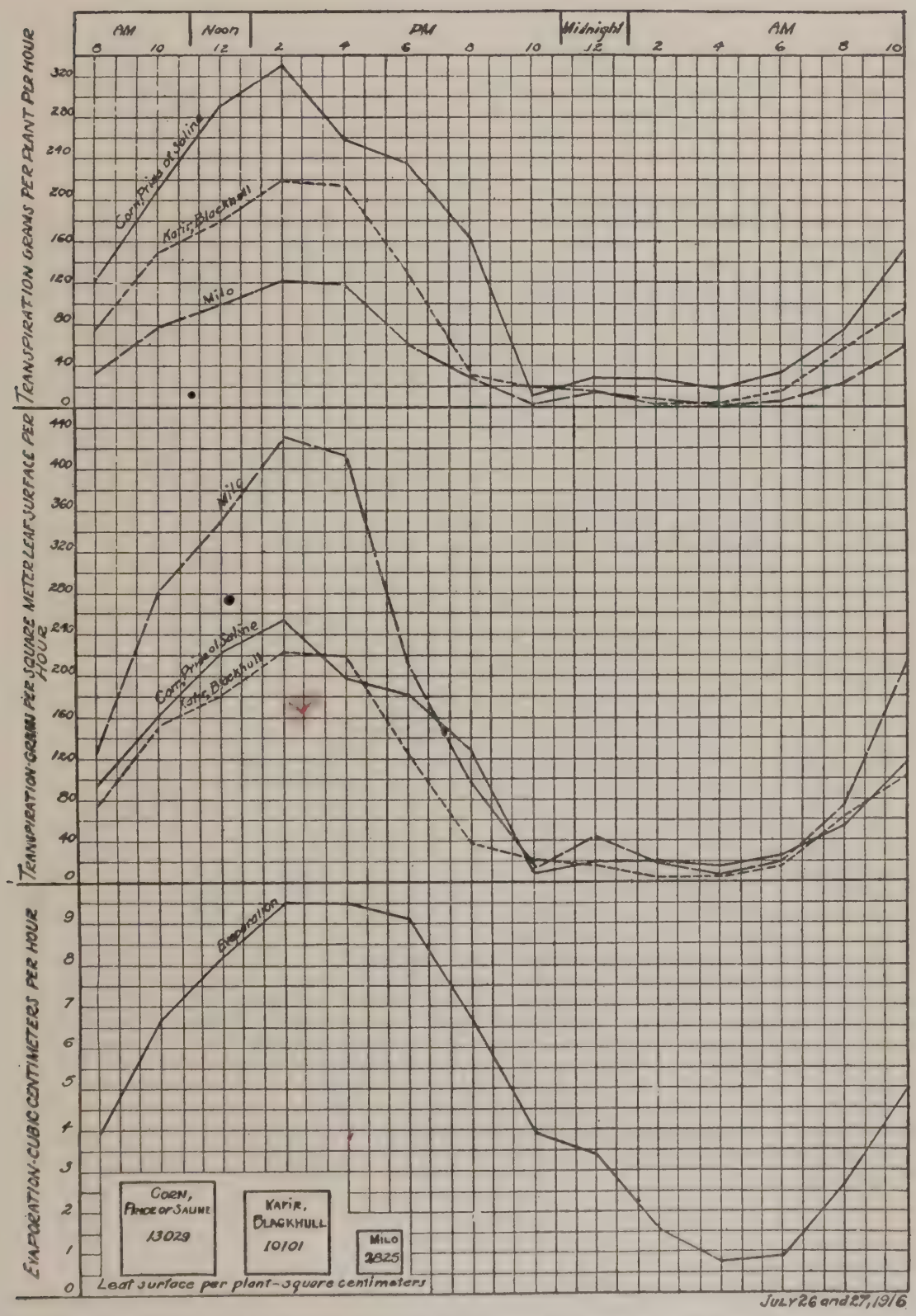

FIG. 4.—Graphs showing the amount of water transpired by Pride of Saline corn, Blackhull kafir, and Dwarf milo during July 26 and 27, 1916, and the evaporation during the corresponding period.

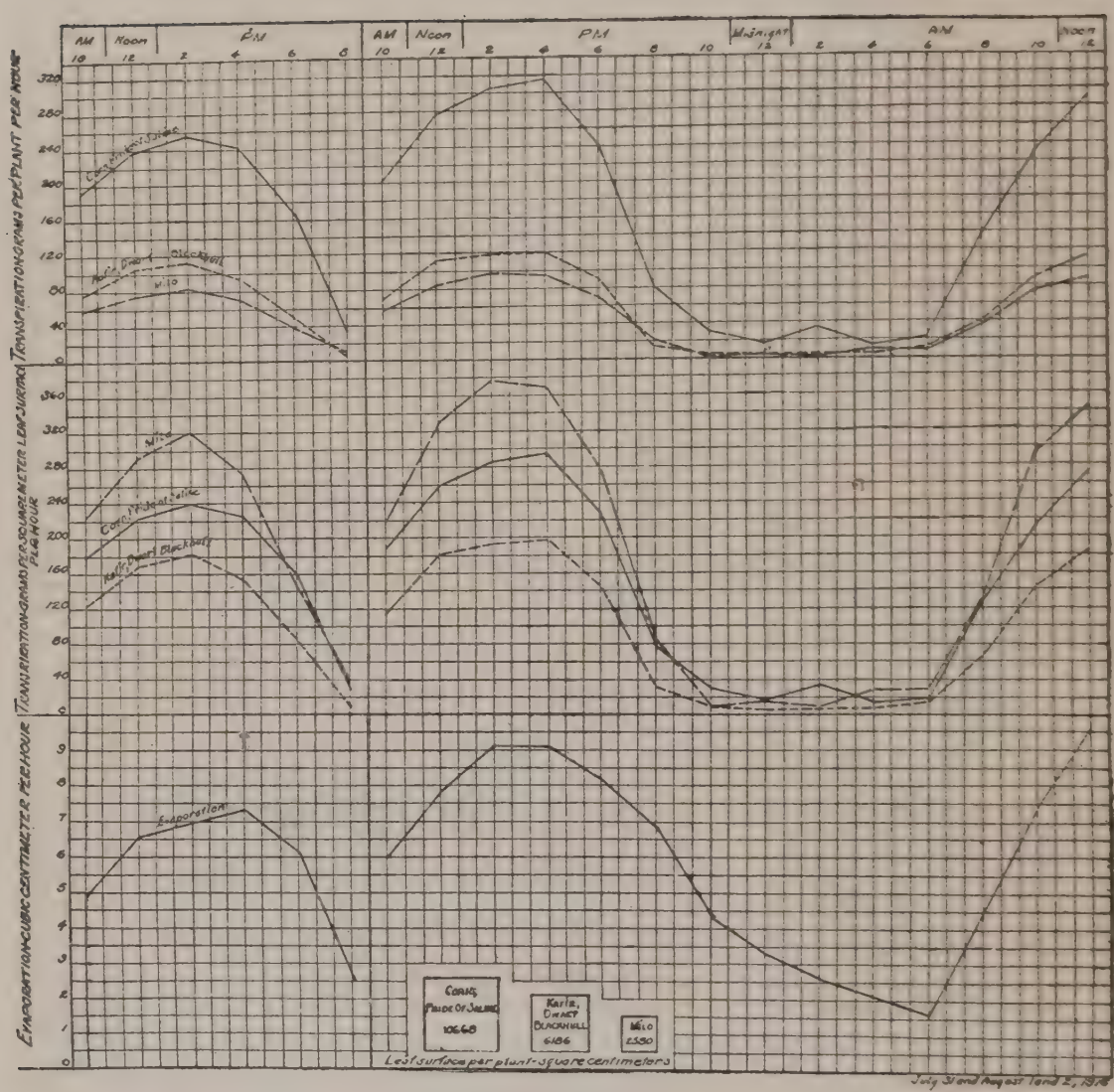

FIG. 5.—Graphs showing the amount of water transpired by Pride of Saline corn, Dwarf Blackhull kafir, and Dwarf milo during July 31 and August 1 and 2, 1916, and the evaporation during the corresponding period.

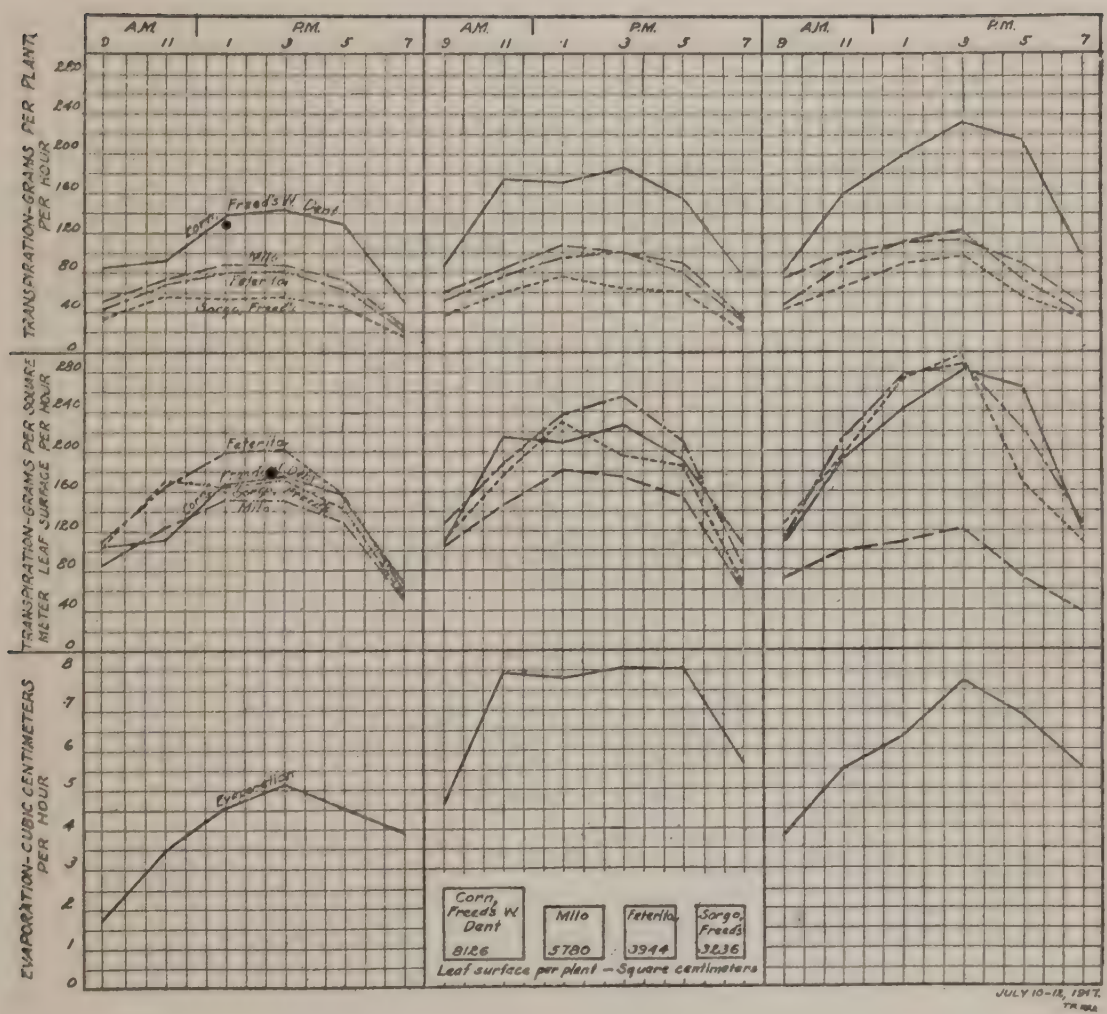

FIG. 6.—Graphs showing the amount of water transpired by Freed's White Dent corn, Dwarf milo, feterita, and Freed's sorgo during July 10, 11, and 12, 1917, and the evaporation during the corresponding period.

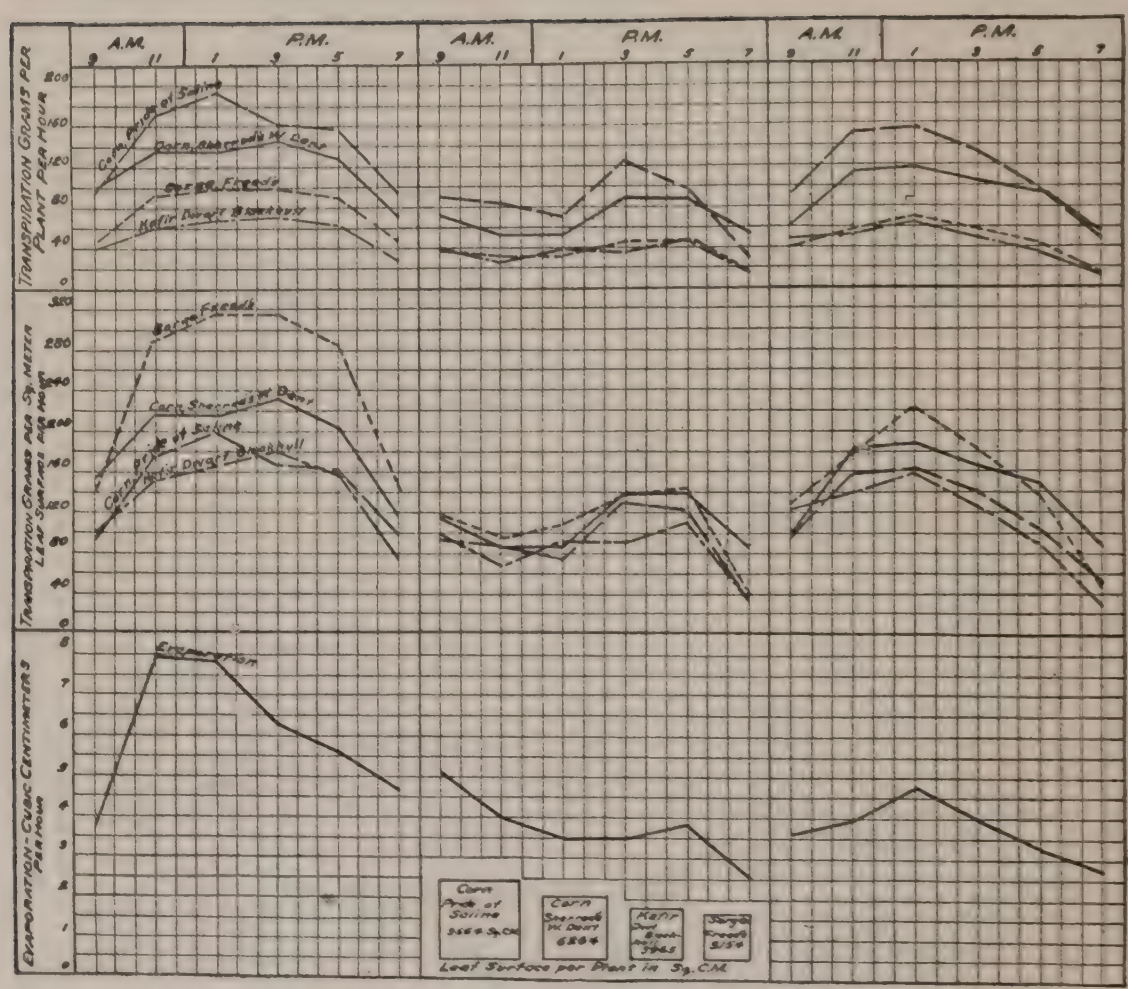

FIG. 7.—Graphs showing the amount of water transpired by Pride of Saline corn, Sherrod's White Dent corn, Dwarf Blackhull kafir, and Freed's sorgo during July 13, 14, and 15, 1917, and the evaporation during the corresponding period.

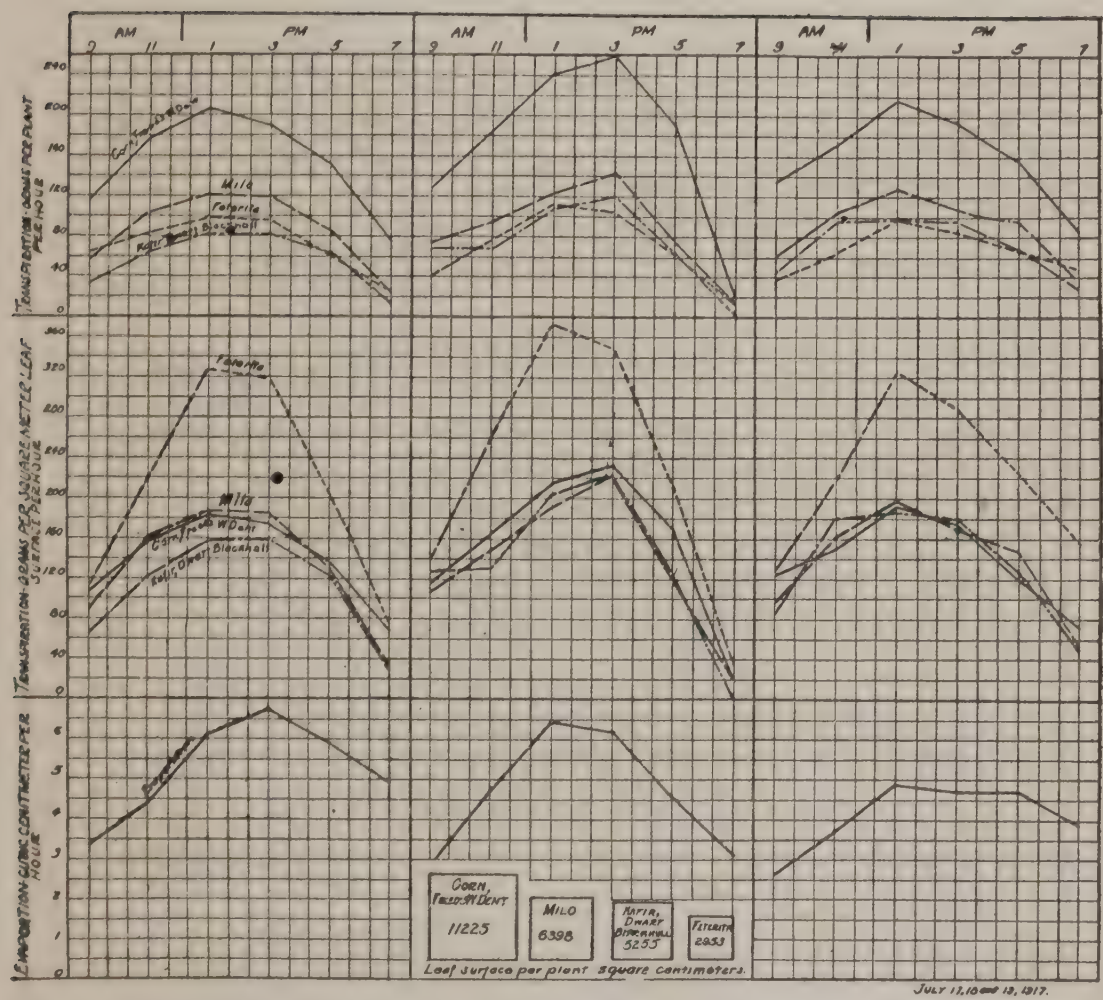

FIG. 8.—Graphs showing the amount of water transpired by Freed's White Dent corn, Dwarf milo, Dwarf Blackhull kafir, and feterita during July 17, 18, and 19, 1917, and the evaporation during the corresponding period.

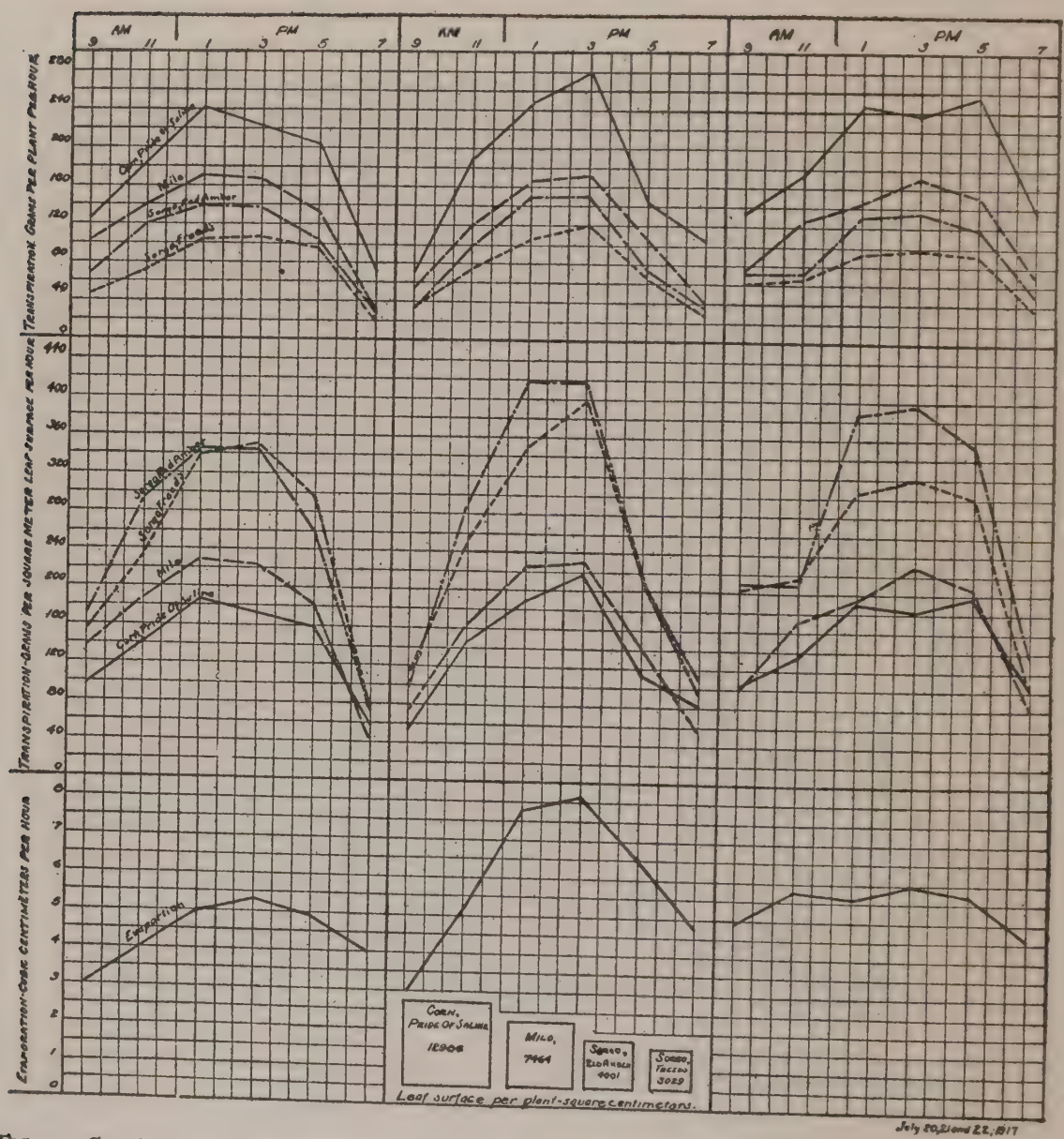

FIG. 9.—Graphs showing the amount of water transpired by Pride of Saline corn, Dwarf milo, Red Amber sorgo, and Freed's sorgo during July 20, 21, and 22, 1917, and the evaporation during the corresponding period.

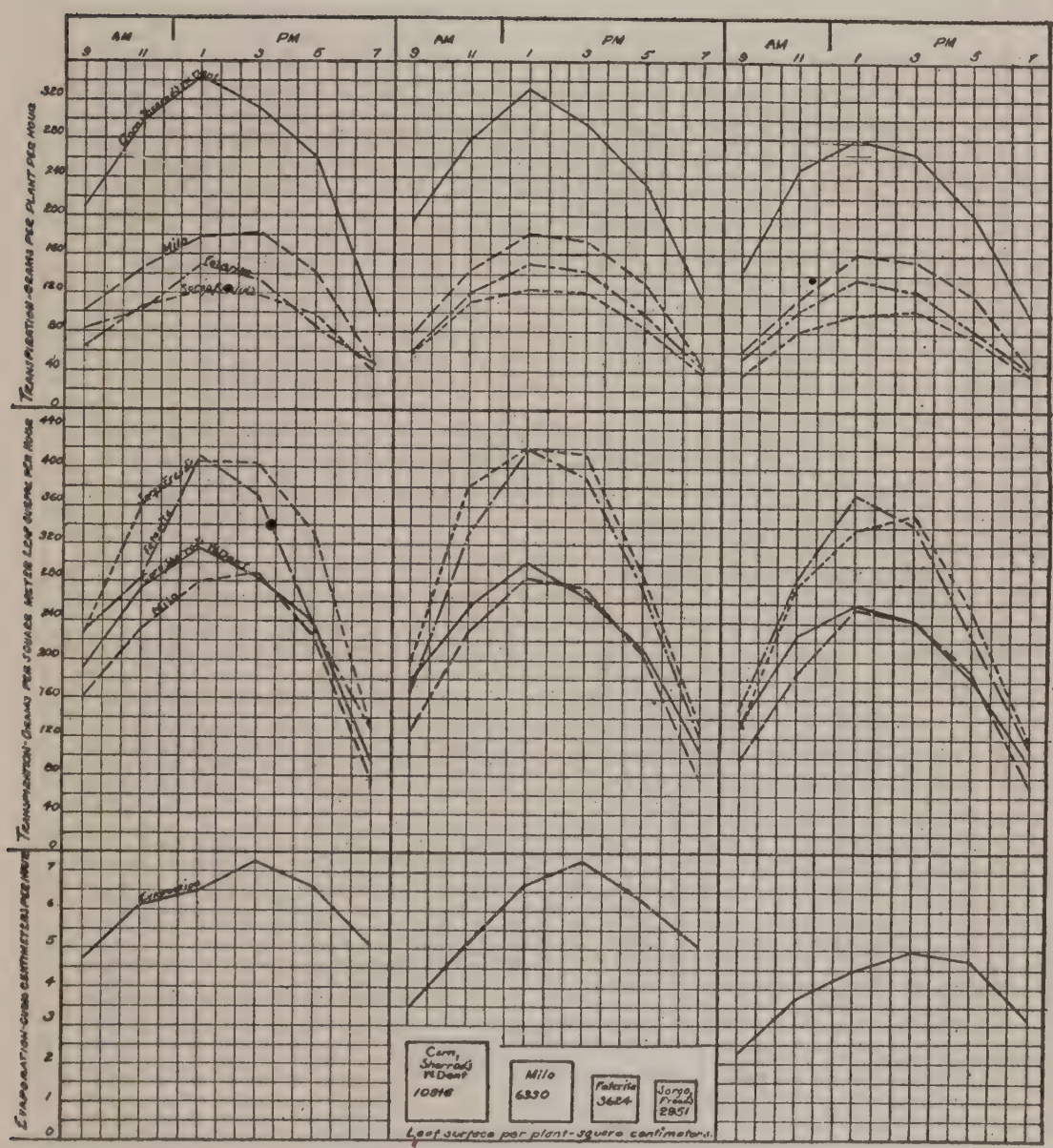

FIG. 10.—Graphs showing the amount of water transpired by Sherrod's White Dent corn, Dwarf milo, feterita, and Freed's sorgo during July 23, 24, and 25, 1917, and the evaporation during the corresponding period.

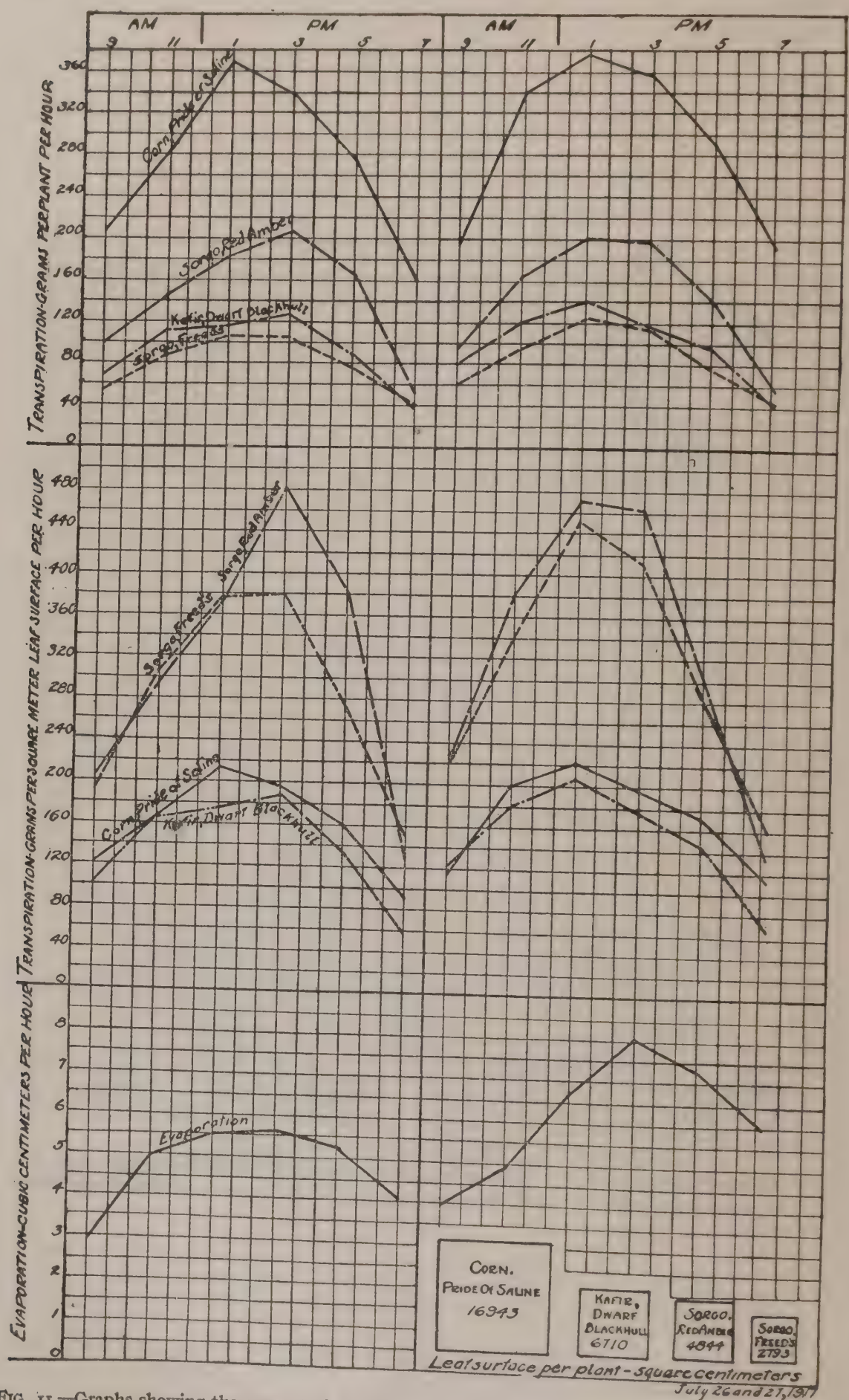

FIG. 11.—Graphs showing the amount of water transpired by Pride of Saline corn, Dwarf Blackhull kafir, Red Amber sorgo, and Freed's sorgo during July 26 and 27, 1917, and the evaporation during the corresponding period.

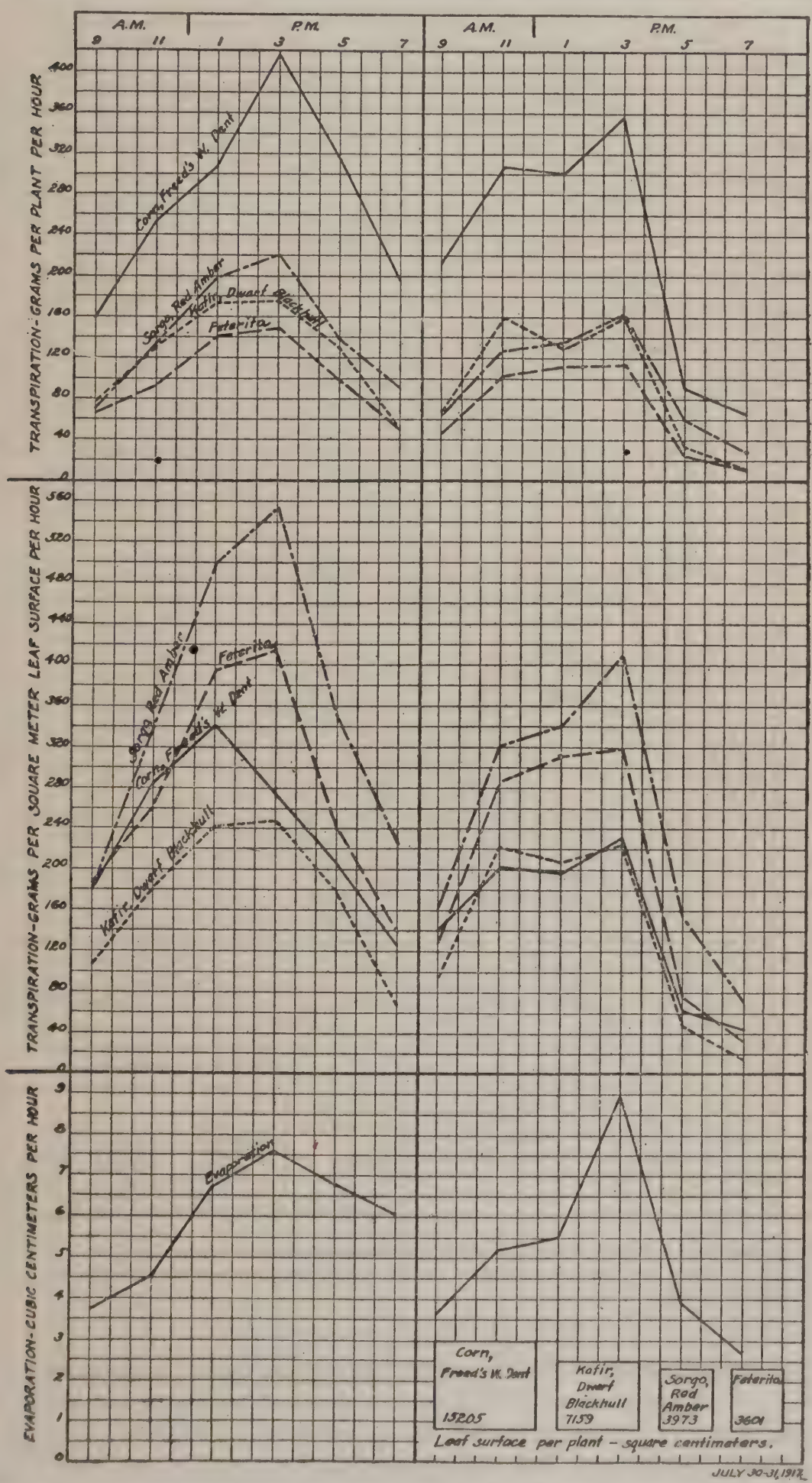

FIG. 12.—Graphs showing the amount of water transpired by Freed's White Dent corn, Dwarf Blackhull kafir, Red Amber sorgo, and feterita during July 30 and 31, 1917, and the evaporation during the corresponding period.

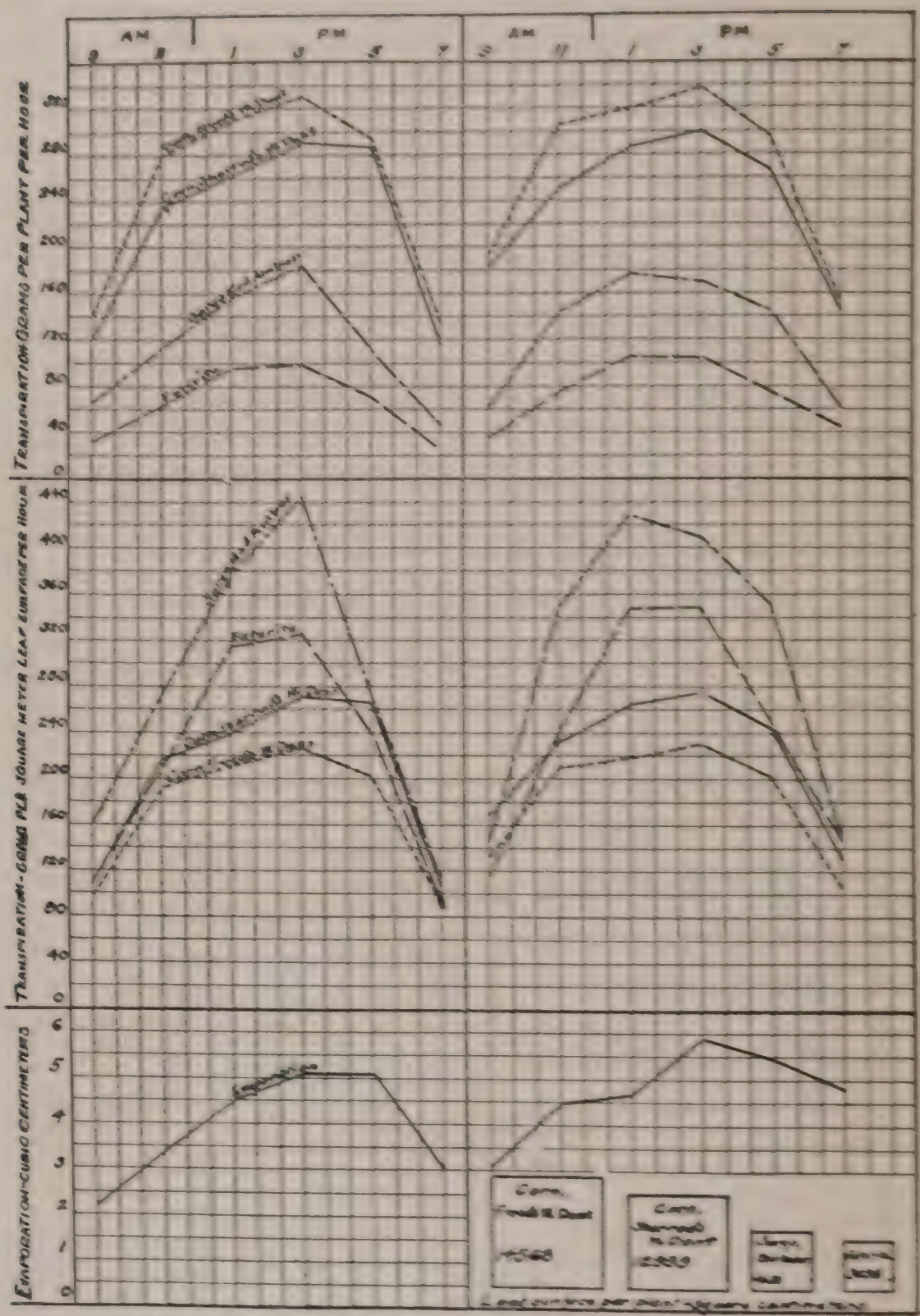

FIG. 13.—Graphs showing the amount of water transpired by Freed's White Dent corn, Sherrod's White Dent corn, Red Amber sorgo, and kafir in August 1 and 3, 1917, with the evaporation during the corresponding period.

LOSS OF WATER PER PLANT

The amount of water transpired by each plant stood in the same relative order as the amount of leaf surface exposed, with the exception of Dwarf Blackhull kafir in 1917. These exceptions of Dwarf Blackhull kafir were observed in the experiments of July 13, 17, 26, and 30 of that year and can be studied from the graphs representing these experi-

ments in figures 7, 8, 11, and 12. Only one of these exceptions will be mentioned here. The leaf surface of the plants used in the experiment of July 26 and 27, 1917, was 16,943, 6,710, 4,844 and 2,793 square centimeters, respectively, for Pride of Saline corn, Dwarf Blackhull kafir, Red Amber sorgo, and Freed's sorgo. The total amount of water transpired per plant during the two days from 7 a. m. to 7 p. m. was 3,353 gm. for Pride of Saline corn, 1,702 gm. for Red Amber sorgo, 1,154 gm. for Dwarf Blackhull kafir, and 989 gm. for Freed's sorgo.

Although in most cases the amount of water transpired by each plant stood in the same relative order as the extent of leaf surface, the loss of water from each plant was not proportional to the amount of leaf surface exposed. For examples of this fact the results of the experiments of July 20, 27, 30, and August 31, 1917, are shown in Table IV. In this table the ratio of the extent of the leaf surface of each of the sorghums to that of the corn plant is shown, together with the ratio of the water loss of each of the sorghum plants to that of corn for every 2-hour period of the day from 7 a. m. to 7 p. m. From these examples it is observed that the ratio of the water loss from the sorghum plants at any given period to that of the corn was higher than the ratio of the ex-extent of leaf surface of these two types of plants. The only exception to this was the Dwarf Blackhull kafir. The ratio of the amount of water transpired by this plant to the amount transpired by the corn plant is lower than the ratio of the extent of the leaf surface of the kafir to that of the corn.

TABLE IV.—*Relation of the amount of water transpired by corn and the sorghums to the extent of the leaf surface of these plants*

Period ending—	Ratio of—					
	Dwarf milo to Pride of Saline corn.		Red Amber sorgo to Pride of Saline corn.		Freed's sorgo to Pride of Saline corn.	
	Leaf surface.	Loss of water per plant.	Leaf surface.	Loss of water per plant.	Leaf surface.	Loss of water per plant.
1917.						
July 20:						
9 a. m.	0.578	0.814	0.310	0.540	0.234	0.370
11 a. m.	.578	.773	.310	.646	.234	.392
1 p. m.	.578	.705	.310	.572	.234	.427
3 p. m.	.578	.748	.310	.618	.234	.475
5 p. m.	.578	.655	.310	.500	.234	.461
7 p. m.	.578	.422	.310	.394	.234	.267
	Dwarf Blackhull kafir to Pride of Saline corn.		Red Amber sorgo to Pride of Saline corn.		Freed's sorgo to Pride of Saline corn.	
July 27:						
9 a. m.	0.396	0.414	0.285	0.484	0.164	0.313
11 a. m.	.396	.358	.285	.479	.164	.282
1 p. m.	.396	.372	.285	.537	.164	.335
3 p. m.	.396	.353	.285	.587	.164	.341
5 p. m.	.396	.333	.285	.487	.164	.268
7 p. m.	.396	.230	.285	.298	.164	.240

TABLE IV.—*Relation of the amount of water transpired by corn and sorghums to the extent of the leaf surface of these plants*—Continued.

Period ending—	Ratio of—					
	Dwarf Blackhull kafir to Freed's White Dent corn.		Red Amber sorgo to Freed's White Dent corn.		Feterita to Freed's White Dent corn.	
	Leaf surface.	Loss of water per plant.	Leaf surface.	Loss of wate per plant.	Leaf surface.	Loss of water per plant.
July 30: 1917.						
9 a. m.	0.471	0.477	0.261	0.446	0.236	0.421
11 a. m.	.471	.505	.261	.529	.236	.368
1 p. m.	.471	.566	.261	.649	.236	.465
3 p. m.	.471	.418	.261	.526	.236	.356
5 p. m.	.471	.406	.261	.438	.236	.276
7 p. m.	.471	.256	.261	.456	.236	.256
	Sherrod's White Dent to Freed's White Dent corn.		Red Amber sorgo to Freed's White Dent corn.		Feterita to Freed's White Dent corn.	
August 3:						
9 a. m.	0.892	0.947	0.283	0.314	0.208	0.183
11 a. m.	.892	.826	.283	.475	.208	.242
1 p. m.	.892	.899	.283	.554	.208	.332
3 p. m.	.892	.884	.283	.504	.208	.314
5 p. m.	.892	.904	.283	.493	.208	.258
7 p. m.	.892	.934	.283	.394	.208	.289

RATE OF TRANSPIRATION

Since the loss of water from the different plants during a given period is not proportional to the extent of their leaf surfaces, it follows that the rate of transpiration per unit of leaf surface is not the same for each plant. Both the Blackhull and the Dwarf Blackhull kafirs showed the lowset transpiration rate per unit of leaf surface in all the experiments in which these plants were used. The corn plant always showed the greatest loss of water per plant, but in all the experiments, with the exception of those in which kafir was used, it showed the lowest rate of water loss per unit of leaf surface. The rate of transpiration per unit of leaf surface in any given experiment was always higher for Dwarf milo Freed's sorgo, Red Amber sorgo, and feterita than for any of the three varieties of corn that were used. In many cases the rate of transpiration for these plants was twice as high as that for corn under the same conditions. The rate of transpiration of the different plants expressed in grams per square meter of leaf surface per hour can be studied from the graphs in figures 1 to 13.

The foregoing discussion shows that the rate of transpiration per unit of leaf surface was, with the exception of the two kafirs, higher for the sorghums than for corn. This difference in transpiration rate became more marked as the leaf surface of the corn plant increased over that of the sorghums. At the earlier stages of growth, when the difference between the extent of leaf surface of these plants was small, the rate of

transpiration per unit of leaf surface was practically the same as that of the sorghums. At the later stages of growth, however, when the plants had attained their full leaf development, the rate of transpiration per unit of leaf surface was often twice as high for the sorghums as for the corn plant under the same conditions. The differences between the rate of transpiration of the corn and sorghum plants is more marked when the plants are under severe climatic conditions than when they are under more favorable environments. This was noted in the differences between the transpiration rate per unit of leaf surface of these plants during the earlier and later periods of the day as compared to those periods of the day when the evaporating power of the air was high.

The results of these experiments with corn and the sorghums seem to indicate that in most cases a small leaf surface is the most important factor in reducing the loss of water from these plants. The corn, with its large extent of leaf surface as compared to the sorghums, always transpires more water per plant in a given period than the sorghums. It is not able, however, to absorb water from the soil and to transport it to so large a leaf surface in sufficient amount to satisfy the evaporating power of the air. As a result of this deficiency, the rate of transpiration per unit of leaf surface is lower than it would be if water were supplied in sufficient quantity to meet the demands of the evaporating power of the air. The sorghums, on the other hand, have, as compared to the corn, a much smaller leaf surface and always transpire less water per plant during any given period than the corn. Since the sorghums have such a small leaf surface exposed for the evaporation of water and since they have a more elaborate root system than the corn, they are able to absorb water from the soil and transport it to the leaves in sufficient amount to satisfy the evaporating power of the air. As a result of this, their rate of transpiration per unit of leaf surface is higher than that of the corn plant.

SUMMARY

Five experiments were conducted in 1916 and eight in 1917 to determine the relative transpiration of corn and the sorghums. Pride of Saline corn, Blackhull kafir, Dwarf Blackhull kafir, and Dwarf milo were used in 1916; and in 1917, in addition to these, Freed's White Dent corn, Sherrod's White Dent corn, Freed's sorgo, Red Amber sorgo, and feterita were used. The plants were grown in large galvanized-iron cans with a capacity of about 120 kilos of soil. The soil used in 1916 had a water content of 18 per cent and a wilting coefficient of 11.1, while the moisture content of the soil used in 1917 was 22 per cent and had a wilting coefficient of 15.1. The moisture content of the soil was kept as nearly constant as possible by the addition of water to the cans from three to four times each day during the time of the experiments. The number of plants grown in each can was reduced to one plant for each of the varieties of

corn, Blackhull kafir, and Red Amber sorgo, while the number of plants of Dwarf milo, feterita, Dwarf Blackhull kafir, and Freed's sorgo varied from one to three to each can.

The transpiration was determined in most of the experiments every two hours from 7 a. m. to 7 p. m. Each experiment extended through two or three days. The cans were weighed on scales of the platform type with a carrying capacity of 180 kilos and were sensitive to about 5 gm. In 1916 the cans were placed in the open on the surface of the ground, but in 1917 they were placed in a pit in the center of a plot that was planted to corn. The pit was of such depth that the tops of the cans were on a level with the surface of the ground.

The amount of water transpired per plant in a given period stood, with the exception of Dwarf Blackhull kafir in 1917, in the same relative order as the extent of leaf surface. The amount of water transpired per plant, however, was not proportional to the extent of leaf surface. Blackhull kafir and Dwarf Blackhull kafir always had the lowest rate of transpiration per unit of leaf surface in the experiments in which these plants were used. All the varieties of corn used always transpired more water per plant during any given period than any of the sorghums. Their rate of transpiration per unit of leaf surface was, with the exception of the kafirs, always much lower than that of the sorghums. The rate of transpiration per unit of leaf surface for feterita, Dwarf milo, Freed's sorgo, and Red Amber sorgo was much higher than that of the corn plant under the same conditions. This difference in the transpiration rate of corn and the sorghums was more marked when the plants had reached their full leaf development and the difference in leaf surface of these plants had reached a maximum. The difference in the transpiration rate per unit of leaf surface was also more evident under severe climatic conditions than under conditions where the evaporation was low.

The results of these experiments with corn and the sorghums seem to indicate that in most cases a small leaf surface is the most important factor in reducing the loss of water from these plants. The corn plant is not capable of supplying its large extent of leaf surface with a sufficient amount of water to satisfy the evaporating power of the air, and as a result its rate of transpiration per unit of leaf surface falls below what it would be if the needed amount of water was supplied. The sorghums, on the other hand, with their small leaf surface are able to supply water in amounts sufficient to satisfy the evaporating power of the air and, as a result, their rate of transpiration per unit of leaf surface is higher than that of the corn.

PLATE 62

A.—Freed's sorgo, Freed's White Dent corn, Dwarf milo, and feterita. July 16, 1917.

B.—Freed's sorgo, Pride of Saline corn, Dwarf Blackhull kafir, and Sherrod's White Dent corn. July 16, 1917.

C.—Dwarf Blackhull kafir. Height of plants, 3 feet. July 30, 1917.

D.—Position of the plants in the field.

E.—Red Amber sorgo and Sherrod's White Dent corn. July 25, 1917.

A
B
C
D
E
19

A
B
C
D

PLATE 63

A.—Blackhull kafir, 4 feet high, heading. Used for transpiration experiments in 1916.

B.—Pride of Saline corn at period of full leaf development. Height of plants, 6 feet. Used for transpiration experiments in 1916.

C.—Dwarf milo heading. Plants 3 feet 3 inches high. July 24, 1917.

D.—Dwarf milo, Pride of Saline corn, and Dwarf Blackhull kafir. July 26, 1916.

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INORGANIC COMPOSITION OF A PEAT AND OF THE PLANT FROM WHICH IT WAS FORMED

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INTRODUCTION

The comparison of the inorganic composition of a peat with that of the material from which it was formed can seldom be made with any certainty, because generally several species of plants have contributed to the deposit. The extensive saw-grass peat deposits found in the Everglades of Florida offer an unusual opportunity to make this comparison, since all indications point to their having been formed by the accumulation of the remains of a single species of plant, saw grass (*Cladium effusum*).

DESCRIPTION AND COMPOSITION OF SAW GRASS

Saw grass is a member of the sedge family and is found chiefly along the banks of streams or ponds and in swamps throughout the southeastern States. By far the largest colony is found in the Everglades, where many thousand acres are covered with an almost impenetrable growth, which reaches a height of 8 to 10 feet in many places. Saw grass resembles ordinary grasses in appearance and derives its name from the fact that the edges and back of the midrib of the leaves are serrated.

On account of the difficulty experienced in obtaining entire plants for analysis, separate samples of leaves, root crowns, and roots were gathered, care being taken to remove all extraneous material. In ashing the samples an electric muffle furnace, fitted with an automatic temperature control set at 550° C., was used to prevent the loss of alkalies by volatilization.

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The determination of silica was made according to the method of the Association of Official Agricultural Chemists,[1] the figure stated thus representing silica of constitution only. Phosphoric acid and the alkalis were determined in the usual manner, and the remaining constituents by the modified Glaser method described by Mellor.[2] The significance of separate values for iron and aluminium oxids was not deemed of importance, and therefore they are reported together. A test was made for titanium, but it was not found in any part of the saw-grass plant. The results of the analyses are given in Table I.

TABLE I.—*Composition of saw grass*

Constituent.	Percentage of dry material.			
	Leaves.	Root crowns.	Roots.	Average.
Silica (SiO_2)	0.50	0.32	0.10	0.30
Iron oxid (Fe_2O_3) and alumina (Al_2O_3)	.09	.08	.16	.11
Lime (CaO)	.59	.59	.52	.57
Magnesia (MgO)	.12	.12	.10	.11
Soda (Na_2O)	.27	.06	.14	.16
Potash (K_2O)	.39	.31	.35	.35
Phosphoric acid (P_2O_5)	.08	.09	.05	.07
Nitrogen	.90	.89	.62	.80

It will be noted that there is comparatively little variation in the composition of the three parts of the plant, with the possible exception of the silica content, and therefore the average of the values found for the three parts is taken as the composition of the saw grass for the subsequent comparisons. The values given are very near the true ones, even in the case of the silica, since it is probable that leaves and roots contribute in nearly equal amounts in the formation of peat.

DESCRIPTION AND COMPOSITION OF PEAT

The peat deposit in the Everglades resulting from the accumulation of the remains of saw grass and varying in depth to about 10 feet, covers a vast area. Near the surface the peat is brown in color, has a loose, fibrous structure, and is very light when dry. It has an ash content of from 7 to 10 per cent. At depths below the surface the material becomes darker in color and less fibrous in texture as the depth increases, grading finally into a black, plastic, compact mass almost free from plant fiber. The ash content also increases slightly with the depth, but even at depths of 6 or 7 feet no free mineral matter can be detected, except in limited

[1] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107, p. 22. 1907.

[2] MELLOR, J. W. A TREATISE ON QUANTITATIVE INORGANIC ANALYSIS. p. 606–609. London, Philadelphia, 1913.

areas where extraneous sand or calcium carbonate contaminate the material.

The samples used in this investigation are representative of about 12 taken from widely separated areas and can be looked upon as being typical of the peat deposit in the Everglades. Sample 1 was taken 9 miles from Lake Okeechobee near the North New River Canal at a depth of 0 to 60 inches. No. 1a is the subsoil of No. 1, its depth being 60 to 120 inches. Sample 2 was taken 20 miles from Lake Okeechobee, a few miles from the North New River Canal, at a depth of 0 to 25 inches, while No. 2a, its subsoil, represents the depth from 25 to 82 inches.

Analyses of this peat were made by practically the same methods used in the case of the ash of the saw grass. The results obtained are presented in Table II.

TABLE II.—*Composition of Everglades peat*

Constituent.	Percentage of dry material.			
	Peat 1.	Peat 2.	Peat 1a (subsoil of 1).	Peat 2a (subsoil of 2).[a]
Silica	1.95	2.04	1.98	3.02
Iron oxid and alumina	.69	.60	.93	1.12
Lime	3.02	2.74	4.67	4.27
Magnesia	.43	.44	.45	.70
Soda	.14	.19	.19	.25
Potash	.11	.06	.09	.06
Phosphoric acid	.13	.15	.07	.07
Nitrogen	3.32	3.84	2.83	2.75
Total ash	7.7	6.8	8.8	10.2

[a] This sample contained a small amount of extraneous sand.

COMPARISON OF PEAT AND SAW-GRASS ANALYSES

In comparing a soil with its parent substance in order to determine the amount of the various constituents removed during the transformation it must be assumed that one of the elements present was not removed at all, or at any rate to a slight extent only. Thus, Merrill [1] compares a granite rock with its resultant clay and assumes that the alumina content was unaltered, while Penrose [2] in comparing a limestone with its resultant clay assumes that no silica was lost during the change. In the present instance the silica was used as the basis of calculation, because it is undoubtedly the most stable constituent present under the conditions of peat formation—that is, continual submersion in water. Table III shows the relationship between the peat and the saw grass when the latter is calculated to the same silica content.

[1] MERRILL, G. P. WEATHERING OF MICACEOUS GNEISS IN ALBEMARLE COUNTY, VIRGINIA. *In* Bul. Geol. Soc. Amer., v. 8, p. 160. 1897.

[2] PENROSE, R. A. F. MANGANESE; ITS USES, ORES, AND DEPOSITS. Ann. Rpt. Geol. Survey Ark. 1890, v. 1, p. 179. 1891.

TABLE III.—*Comparison of the composition of peat and saw grass*

Constituent.	Peat 1.	Saw grass × silica ratio (6.5).	Percentage loss.	Peat 2.	Saw grass × silica ratio (6.8).	Percentage loss.
Silica	1.95	1.95	0.0	2.04	2.04	0.0
Iron oxid and alumina	.69	.72	4.2	.60	.75	20.0
Lime	3.02	3.70	18.4	2.74	3.88	29.3
Magnesia	.43	.72	40.3	.44	.75	41.3
Soda	.14	1.04	86.5	.19	1.09	82.6
Potash	.11	2.27	95.1	.06	2.38	97.5
Phosphoric acid	.13	.45	71.1	.15	.48	68.7
Nitrogen	3.32	5.20	36.1	3.84	5.44	29.4

The losses obtained for the two samples of surface peat cited agree very well except in the case of the iron oxid and alumina, and even here the variation is not excessive. No comparison was made with the subsoil peats because they have been subjected to the leaching action of water so long that more or less of all the elements present must have been removed. In the subsoil 1a there appears to have been a considerable loss of silica, while both samples contain much less nitrogen than those taken from nearer the surface. However, this is to be expected when it is considered that hundreds, if not thousands, of years have elapsed since the deposition of these lower strata.

The comparatively small loss of lime suffered by the saw grass may seem surprising at first, and it is unusual, as this constituent is leached very readily from ordinary soils. There are two possible explanations for its behavior under the circumstances. In the first place, the lime is present in the plant for the most part as difficultly soluble compounds (calcium oxalate, etc.), and in the second place, owing to the great abundance of calcium carbonate in the Everglades, the solvent action of the waters upon the lime in the peat must be far less than it would be under other conditions.

In the formation of ordinary soils, potash is held by adsorption, and, hence, suffers a lower percentage loss than some of the less soluble elements; but this constituent is leached very readily from leaves and vegetation,[1] and nearly the entire amount originally present in the saw grass has been removed in the transformation to peat.

The enormous accumulation of nitrogen in the peat is an interesting phase of the change undergone by the saw grass. The loss of nitrogen seems to be very gradual, and even in the subsoil there is still a very high percentage of this element. The prevailing poor conditions for bacterial activity while the peat is being formed both preclude the possibility of nitrogen fixation, and also account for the greater stability of the nitrogen in the peat than in ordinary soils, wherein the nitrogen is oxidized to nitrate and thus leached.

[1] LE CLERC, J. A., and BREZEALE, J. F. PLANT FOOD REMOVED FROM GROWING PLANTS BY RAIN OR DEW. *In* U. S. Dept. Agr. Yearbook, 1908, p. 389-402. 1909.

In Table IV a comparison is made of the losses suffered by three widely differing soil-forming materials in their transformation to soils. As previously stated, the behavior of the lime in the saw grass is strikingly different from that in the other cases cited and considerably more potash also was removed.

TABLE IV.—*Comparison of the losses of three soil-forming materials in their transformation to soils*

Constituent.	Percentage losses suffered by parent material in soil formation.		
	Arkansas limestone (8 parts yield 1 of soil).[a]	Granite (1.7 parts yield 1 of soil).[b]	Saw grass (7 parts yield 1 of peat).
Silica	0.00	52.45	0.00
Iron oxid	89.56	14.35	12.2
Alumina	11.35	00.00	
Lime	98.93	100.00	23.8
Magnesia	89.38	74.70	40.8
Potash	66.36	83.52	96.3
Soda	53.26	95.03	84.6
Nitrogen	.....	.....	32.8

[a] PENROSE, R. A. F. LOC. CIT. [b] MERRILL, G. P. LOC. CIT.

CONCLUSIONS AND SUMMARY

In this article the inorganic composition of typical samples of Everglades peat is given together with analyses of the parent material from which the peat was formed—namely, saw grass (*Cladium effusum*). Brief descriptions of both products are also given. Assuming that no silica was lost during the transformation, about 7 parts of saw grass were required to yield 1 of peat. Based on this assumption, the constituents were leached to the following extent:

Iron oxid and alumina, 12.2 per cent; lime, 24 per cent; magnesia, 41 per cent; potash, 96 per cent; soda, 84.6 per cent; phosphoric acid, 70 per cent; and nitrogen, 33 per cent. The losses suffered by two other common soil-forming substances, granite and limestone, are shown for the sake of comparison.

DIGESTIBILITY OF CORN SILAGE, VELVET-BEAN MEAL, AND ALFALFA HAY WHEN FED SINGLY AND IN COMBINATIONS

By P. V. Ewing, *Animal Husbandman, Texas Agricultural Experiment Station* and F. H. Smith,[1] *Georgia Agricultural Experiment Station*

INTRODUCTION

This paper is the third in a series of investigations on the associative action of various feeds. The results with rations made up of corn silage, cottonseed meal, and starch [2] and those made up of corn silage and cottonseed meal [3] have been published, and the present article deals with the question of the digestibility of rations made up of corn silage, velvet-bean meal, and alfalfa hay. The investigation was conducted with the view of studying the extent and causes of fluctuations in the total nutrient digestibility induced by the combinations of these feeds, and the relationship of these fluctuations to the feeding practice.

PLAN OF INVESTIGATION

The steers used were the high-grade 2-year-old north-Georgia Shorthorns which had been used in the nutrition work of the winter of 1915–16 (previously reported).[4]

The corn silage and alfalfa hay were both produced on the Station farm and were of average grade. The velvet-bean meal was from commercial stock and came from near the southern Georgia-Alabama line. The average analyses of these feeds are given in Table I.

Table I.—*Average percentage composition of the feeds used*

Feed.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
Silage	26.29	0.773	7.304	1.180	0.252	15.46
Alfalfa hay	91.02	2.675	30.770	6.865	2.564	34.68
Velvet-bean meal	89.37	4.576	14.430	4.250	2.764	48.84

[1] F. H. Smith is now a lieutenant in the Aviation Branch of the United States Army. The work on which this paper is based was done while the senior and junior writers were connected with the Georgia Agricultural Experiment Station in the capacities of Animal Husbandman and Chemist, respectively.

[2] Ewing, P. V., and Wells, C. A. THE ASSOCIATIVE DIGESTIBILITY OF CORN SILAGE, COTTONSEED MEAL, AND STARCH IN STEER RATIONS. Ga. Agr. Exp. Sta. Bul. 115, p. 269–296, 7 diagr. 1915.

[3] Ewing, P. V., Wells, C. A., and Smith, F. H. THE ASSOCIATIVE DIGESTIBILITY OF CORN SILAGE AND COTTONSEED MEAL IN STEER RATIONS. pt. 2. Ga. Agr. Exp. Sta. Bul. 125, p. 149–164, 1 fig. 1917.

[4] Ewing, P. V., and Smith, F. H. A STUDY OF THE RATE OF PASSAGE OF FOOD RESIDUES THROUGH THE STEER AND ITS INFLUENCE ON DIGESTION COEFFICIENTS. *In* Jour. Agr. Research, v. 10, no. 2, p. 55–63. 1917.

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Nine different rations were fed, and the digestion trials were made in triplicate, different quantities of the feeds being used in each of the three trials. The first three rations contained but one feed each, the next three contained two feeds each, and the last three contained three feeds each. A feature of the rations was the variations presented as to quantities and proportions, and in each of the last three rations the quantity in one feed was varied, while the other two were kept constant. This provided means for checking the influences exerted by the specific components of the rations. The composition of the various rations and the weight of the feces excreted daily are given in Table II. The percentage composition of the feces is given in Table III.

TABLE II.—*Weights of animals, feed, and feces*

Ration No.	Digestion trial No.	Steer No.	Average weight.	Average daily feed.			Average weight of feces excreted daily.
				Silage.	Velvet-bean meal.	Alfalfa	
			Kgm.	*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*
I	a	63	260	0	.....	.....	2.280
	b	64	284	8	.....	.....	4.705
	c	62	250	10	.....	.....	5.045
II	a	61	273	.....	.....	3.000	4.042
	b	66	280	.....	.....	4.000	6.120
	c	65	282	.....	.....	4.202	6.850
III	a	65	270	.....	3.50	.....	2.600
	b	65	288	.....	3.50	.....	2.308
	c	65	277	.....	3.50	.....	2.521
IV	a	60	284	2	.....	4.000	6.951
	b	65	293	3	.....	3.000	6.208
	c	61	278	4	.....	2.000	4.934
V	a	64	298	4	3.50	.....	5.072
	b	63	282	0	2.75	.....	5.749
	c	62	271	8	2.00	.....	6.475
VI	a	64	290	.....	2.00	3.000	8.128
	b	63	274	.....	2.80	2.800	7.745
	c	62	290	.....	3.00	2.000	6.480
VII	a	62	306	8	2.00	2.000	9.950
	b	63	318	6	2.00	2.000	9.193
	c	64	335	4	2.00	2.000	8.372
VIII	a	64	335	4	2.00	2.000	8.372
	b	61	202	4	1.50	2.000	6.367
	c	66	298	4	2.50	2.000	9.217
IX	a	64	335	4	2.00	2.000	8.372
	b	62	318	4	2.00	2.500	7.638
	c	63	318	4	2.00	3.000	8.610

TABLE III.—*Percentage composition of the feces of steers*

Ration No.	Digestion trial No.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
I	a	20.34	0.459	4.115	2.276	0.391	11.05
	b	15.48	.445	3.008	1.714	.313	8.36
	c	15.07	.346	3.113	1.467	.300	8.27
II	a	21.76	.972	8.238	2.677	.507	6.70
	b	21.72	1.035	8.336	2.200	.520	6.90
	c	21.28	.998	8.331	2.291	.464	6.76
III	a	20.50	1.888	4.159	2.818	.983	5.49
	b	19.70	1.086	4.355	2.324	.841	8.68
	c	20.205	1.592	4.231	2.633	.931	6.665
IV	a	24.24	1.097	8.222	2.552	.541	8.99
	b	20.06	.920	6.635	2.221	.437	7.55
	c	21.53	.931	6.668	2.330	.498	8.49
V	a	19.42	.900	4.262	1.641	.628	8.69
	b	18.17	.691	4.373	1.577	.547	8.11
	c	15.60	.444	3.638	1.426	.448	7.29
VI	a	16.97	.965	6.118	1.660	.428	5.55
	b	19.40	.955	6.658	1.812	.565	6.44
	c	18.05	.799	6.218	1.929	.555	5.64
VII	a	17.83	.611	5.787	1.699	.405	7.20
	b	19.50	.727	6.361	1.865	.473	7.59
	c	17.39	.750	5.716	1.779	.455	6.30
VIII	a	17.39	.750	5.716	1.779	.455	6.30
	b	20.23	.800	6.126	2.319	.550	7.55
	c	16.37	.689	4.723	1.916	.454	6.20
IX	a	17.39	.750	5.716	1.779	.455	6.30
	b	19.49	.778	6.215	2.279	.502	7.08
	c	20.08	.811	6.444	2.293	.518	7.30

The equipment used and the general methods of weighing and sampling the feeds were similar to those previously described.[1] The digestion trials were of 12 days' duration each, with a minimum preliminary feeding period of 18 days. The quantity of feed was so gauged that there were no orts, and if a steer failed to eat its feed, the trial with that animal was discontinued.

DISCUSSION OF RESULTS

The digestion coefficients, with which we are primarily concerned, obtained for the several rations are given in Tables IV to X, being summarized and averaged in Tables VIII, IX, and X. In each instance the starting point for these studies is the result of feeding-alone experiments, probably the most accurate guide on the digestion of a specific food, especially when the metabolic products are taken into account. However, this has not been done in this study.

[1] EWING, P. V., WELLS, C. A., and SMITH, F. H. Op. cit.

TABLE IV.—*Digestion coefficients of the total nutrients of each ration*

Ration No.	Digestion trial No.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen free extract.
I	a	70.58	78.26	78.54	26.76	40.00	72.85
	b	65.38	66.13	75.69	13.83	25.00	68.23
	c	71.09	77.92	78.49	37.29	40.00	73.03
II	a	63.02	43.75	58.61	39.81	68.83	70.13
	b	62.67	41.12	58.57	50.91	68.93	69.60
	c	62.68	40.87	56.78	46.78	70.91	68.91
III	a	82.90	69.38	78.42	50.34	73.20	91.63
	b	85.10	83.75	79.61	63.09	79.38	90.05
	c	83.72	75.00	78.81	55.71	75.26	90.17
IV	a	61.31	40.16	60.28	43.14	66.67	64.76
	b	64.12	43.69	63.40	41.91	67.06	68.38
	c	63.02	45.88	63.73	37.50	59.02	68.06
V	a	76.44	75.92	72.90	57.65	70.09	81.05
	b	74.10	76.74	69.94	51.60	65.93	79.48
	c	74.04	81.17	72.97	48.60	61.33	78.68
VI	a	69.48	54.65	58.99	53.61	73.49	77.65
	b	70.24	63.55	59.24	54.99	70.47	78.67
	c	74.01	72.78	61.55	52.83	73.14	83.10
VII	a	68.93	70.67	61.29	46.52	68.25	75.38
	b	65.41	65.10	56.41	41.30	66.94	73.14
	c	68.75	64.41	59.95	44.61	67.24	76.98
VIII	a	68.75	64.41	59.95	44.61	67.24	76.98
	b	69.43	66.88	65.27	40.32	65.69	76.48
	c	70.45	67.84	65.69	38.97	67.69	77.46
IX	a	68.75	64.41	59.95	44.61	67.24	76.98
	b	70.89	68.95	64.82	42.76	70.54	78.03
	c	68.96	65.52	63.10	41.72	68.31	76.14

TABLE V.—*Digestion coefficients of corn silage by difference* [a]

Ration No.	Digestion trial No.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
I	a	70.58	78.26	78.54	26.76	40.00	72.85
	b	65.38	66.13	75.69	13.83	25.00	68.23
	c	71.09	77.92	78.49	37.29	40.00	73.03
IV	a	49.82	26.67	79.57	.....	.....	43.37
	b	68.06	47.83	86.30	18.86	37.50	65.74
	c	63.12	51.61	75.68	12.77	10.00	66.35
V	a	54.18	74.19	62.33	61.70	20.00	54.69
	b	58.78	78.27	61.87	43.66	13.33	63.36
	c	65.62	88.71	70.03	41.49	20.00	69.28
VII	a	61.34	87.10	55.99	38.30	45.00	66.61
	b	47.24	69.57	39.27	14.08	26.67	57.43
	c	52.95	67.75	45.21	19.15	10.00	63.75
VIII	a	52.95	67.75	45.21	19.15	10.00	63.75
	b	62.17	90.32	70.21	2.13	10.00	67.48
	c	54.75	80.64	65.41	.....	10.00	60.36
IX	a	52.95	67.75	45.21	19.15	10.00	63.75
	b	65.87	106.45	68.84	6.38	40.00	70.06
	c	59.03	93.55	63.70	2.13	10.00	64.40

[a] The alfalfa and velvet-bean meal digestion coefficients are based on feeding-alone experiments.

TABLE VI.—*Digestion coefficients of velvet-bean meal by difference* [a]

Ration No.	Digestion trial No.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
III	a	82.90	69.38	78.42	50.34	73.20	91.63
	b	85.10	83.75	79.61	63.09	79.38	90.05
	c	83.72	75.00	78.81	55.71	75.26	90.17
V	a	78.33	75.63	69.70	65.77	73.20	83.97
	b	76.20	76.19	60.45	63.25	71.05	84.06
	c	77.78	83.70	61.94	67.06	69.09	85.98
VI	a	79.41	65.22	62.28	72.94	78.18	86.29
	b	77.66	76.56	61.88	69.75	71.43	85.16
	c	81.50	84.67	66.51	60.16	75.90	89.50
VII	a	72.75	82.61	33.56	63.53	78.18	72.37
	b	63.12	71.74	19.38	41.18	72.73	75.85
	c	73.42	72.83	45.33	49.41	70.91	84.75
VIII	a	73.42	72.83	45.33	49.41	70.91	84.75
	b	77.11	81.16	68.06	34.37	68.29	85.95
	c	76.32	77.28	68.42	33.02	71.01	84.21
IX	a	73.42	72.83	45.33	49.41	70.91	84.75
	b	81.03	85.87	69.20	42.35	76.36	88.74
	c	77.00	81.52	64.01	37.65	70.91	85.16

[a] The corn-silage and alfalfa-hay digestion coefficients are based on feeding-alone experiments.

TABLE VII.—*Digestion coefficients of alfalfa hay by difference* [a]

Ration No.	Digestion trial No.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
II	a	63.02	43.75	58.61	39.81	68.83	70.13
	b	62.67	41.12	58.57	50.91	68.93	69.60
	c	62.68	40.87	56.78	46.78	70.91	68.91
IV	a	64.91	34.58	58.08	44.00	67.96	63.69
	b	62.13	33.75	59.80	43.69	70.12	66.18
	c	58.51	27.77	56.74	39.41	62.74	63.69
VI	a	60.01	30.00	52.76	52.42	71.42	65.51
	b	56.85	42.66	50.00	54.16	65.27	61.77
	c	59.39	64.81	49.26	49.63	68.62	67.29
VII	a	51.92	53.70	36.74	50.36	70.58	58.50
	b	42.52	35.18	30.07	36.49	64.70	48.67
	c	52.63	37.03	42.27	41.60	62.74	61.38
VIII	a	52.63	37.03	42.27	41.60	62.74	61.38
	b	57.96	50.00	54.14	35.77	62.74	64.69
	c	53.68	44.44	51.87	27.73	62.74	58.40
IX	a	52.63	37.03	42.27	41.60	62.74	61.38
	b	60.58	55.23	54.35	38.95	70.31	67.12
	c	58.44	48.75	53.30	37.86	66.23	64.45

[a] The corn-silage and velvet-bean meal digestion coefficients are based on feeding-alone experiments.

TABLE VIII.—*Average corn-silage digestion coefficients when velvet-bean meal and alfalfa-hay coefficients are based on feeding-alone experiments*

Method of feeding.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
Alone	69.02	74.10	77.57	25.96	35.00	71.37
With alfalfa hay	60.33	42.04	80.52	15.82	23.75	58.49
With velvet-bean meal	59.53	80.39	64.74	48.95	17.78	62.44
With alfalfa hay and velvet-bean meal	56.58	81.21	55.45	15.06	19.07	64.18
Average	61.37	69.43	69.57	26.45	23.90	64.12

TABLE IX.—*Average velvet-bean meal digestion coefficients by difference when alfalfa-hay and corn-silage coefficients are based on feeding-alone experiments*

Method of feeding.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
Alone	83.91	76.04	78.95	56.36	75.95	90.62
With corn silage	77.42	78.51	64.03	65.36	71.11	84.67
With alfalfa hay	79.52	75.48	63.03	67.46	75.17	86.98
With corn silage and alfalfa hay	74.17	77.63	50.96	44.48	72.25	82.95
Average	78.76	76.92	64.38	58.42	73.62	86.31

TABLE X.—*Average alfalfa-hay digestion coefficients by difference when velvet-bean meal and corn-silage coefficients are based on feeding-alone experiments*

Method of feeding.	Dry matter.	Ether extract.	Crude fiber.	Ash.	Nitrogen.	Nitrogen-free extract.
Alone	62.79	41.91	57.99	45.83	69.56	69.55
With corn silage	61.85	32.03	58.21	42.37	66.94	64.52
With velvet-bean meal	58.75	45.82	50.67	52.07	68.44	64.86
With corn silage and velvet-bean meal	53.67	33.26	45.25	39.11	65.06	60.66
Average	59.27	38.26	53.03	44.85	67.50	64.90

Reference to the tables show that, as a general proposition, the digestibility of a feed is greatest when fed alone, next greatest when fed in combination with one other feed, and least when fed in combination with the two other feeds. In this connection, however, the apparent loss in digestibility is not necessarily all attributable to the combination, for the total dry matter consumed in the rations containing two feeds was greater than in the rations containing one feed, and the total dry matter of the rations made up of three feeds was greater than the rations containing two feeds. Thus, the quantity of the feed in the larger rations probably had a depressing effect on digestibility. Kellner [1] cites his own experiments with steers fed mixed rations which show an apparent decrease in digestibility with an increasing quantity of feed, and Armsby and Fries [2] state that their results seem to—

> indicate clearly a real, although slight, effect of increasing quantity in diminishing the percentage digestibility.

Our results have shown the same slight decline, but it is not probable that the total decline in digestion coefficients obtained with three feeds compared with two, and with two feeds compared with one, is accounted

[1] KELLNER, OSKAR. DIE ERNÄHRUNG DER LANDWIRTSCHAFTLICHEN NUTZTIERE. Aufl. 6, p. 48. Berlin, 1912.

[2] ARMSBY, H. P.; and FRIES, J. A. THE INFLUENCE OF TYPE AND OF AGE UPON THE UTILIZATION OF FEED BY CATTLE. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 128, p. 28. 1911.

for by the increased quantity of feed. We must therefore conclude that in general, so far as the feeds we are studying are concerned, the combination with other feeds has a depressing effect on digestion. This does not necessarily follow for all the individual nutrients.

It is evident from a study of the tables that, so far as the average apparent digestion coefficients of silage are concerned, nothing is especially abnormal or noteworthy. It is true that the nitrogen (crude protein) digestion presents some irregularities when fed in combination with other feeds, but when fed alone these irregularities are not out of the ordinary. The amount of nitrogen in corn silage is so small that variations are likely to occur. Ash digestion also not only shows considerable irregularity at times but frequently shows a negative coefficient. This same condition has been noted in previous digestion experiments.

The apparent coefficients obtained for velvet-bean meal are of special interest on account of the relatively few digestion trials that have been made with this comparatively important feed. From our results it is seen that, as a whole, this feed is rather highly digestible; and when fed alone approximately 84 per cent of the dry matter is digested. The apparent digestibility of none of the nutrients averaged less than 58 per cent.

The apparent digestion coefficients for alfalfa hay present no marked irregularities. They do show the same general decline as a result of combination and quantity of feed. This decline amounts to over 10 per cent from feeding alone to the combining of all three feeds in Ration VII, where the total quantity of dry matter consumed was greater than in any other ration.

Thus, these results show no great variations from what might have been expected. While it is true that from an economic standpoint we are interested in that combination of feeds which will give the greatest digestibility for all the nutrients of the rations, these results do show no marked decline as a result of food combinations. If marked declines existed, their presence would be magnified by the determination of digestion coefficients by difference, but here we have no marked variations from the normal and can therefore conclude that the combining of these feeds has resulted in no marked increase or decrease in apparent digestion coefficients.

When rations that contain silage, velvet-bean meal, and alfalfa hay are fed, the tendency seems to be to lower the digestibility of the whole slightly below the calculated digestion coefficients. There are indications also that slightly better use is made of the feeds fed when the ration is made up of silage and one of the protein feeds than when both the protein feeds are fed, and that the most complete digestion takes place with silage and alfalfa rather than with the silage and velvet-bean meal, although the difference is small.

We appreciate the effect of individual variation in digestion and the slight variations which are likely to occur as a result of making the digestion trials at different times. The trials were made in triplicate, and the time factor was minimized by the method of planning the experiment. The slight variations noted are so general that it is hardly conceivable that they could in any way be accounted for by the possible errors mentioned.

CONCLUSIONS

Aside from the actual digestion coefficients obtained by the feeding of these feeds alone and under the different combinations, a review of the coefficients seems to justify the following conclusions:

(1) The combining of these feeds in general tends toward lowering the digestibility of the several nutrients of the rations.

(2) The digestion of corn silage, alfalfa hay, and velvet-bean meal is apparently fairly constant under the different combinations.

(3) More accurate digestion coefficients are obtained by feeding-alone experiments, where such are possible, rather than by the usual difference method.

(4) Greater variations are presented in the apparent digestibility of nitrogen and ash than in other nutrients.

(5) Compared with similar feeds, velvet-bean meal is apparently well digested.

EFFECTS OF VARIOUS SALTS, ACIDS, GERMICIDES, ETC., UPON THE INFECTIVITY OF THE VIRUS CAUSING THE MOSAIC DISEASE OF TOBACCO

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In the course of the writer's investigations of the mosaic disease of tobacco (*Nicotiana* spp.) many experiments have been carried out to determine the effects of different concentrations of salts, acids, alkalis, germicides, etc., upon the infectivity of the virus. The object in view was to throw some light on the question of the nature of the causal infective agent and the degree of resistance of this infective principle to the various chemical and germicidal treatments used. In these experiments all concentrations stated as 1 gm. of reagent in 100 c. c., 200 c. c., etc., of solution refer to 1 gm. of actual water-free salt or other reagent in the indicated volume of solution. The method of preparation of the various concentrations has been uniform. For determining the action of various concentrations of these substances, a double-strength concentration was first made with distilled water. Ten c. c. of virus were then added to 10 c. c. of the solution, the concentration being brought down to the strength desired. By using this method of preparation, the virus itself is uniformly diluted to half its original strength in all solutions. As the undiluted stronger concentrations (see Table I) frequently injure the leaf tissues severely, or even kill the plants outright if inoculated into them, it was sometimes necessary to dilute the solutions with several volumes of water at the time the inoculations were made.

The results of the various concentrations of substances used are shown in Table I and text following.

Table I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*

NITRIC ACID

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. to 500 c. c. of virus solution...	June 9, 1914	June 23, 1914 (14 days later).	10 Connecticut Broadleaf.	10 plants mosaic.
1 gm. to 800 c. c. of virus solution...	.....do.....	.....do.....	.....do.....	9 plants mosaic.
1 gm. to 1,000 c. c. of virus solution..	.....do.....	.....do.....	.....do.....	Do.
1 gm. to 1,600 c. c. of virus solution..	.....do.....	.....do.....	.....do.....	10 plants mosaic.
1 gm. to 1,800 c. c. of virus solution..	.....do.....	.....do.....	.....do.....	Do.
1 gm. to 2,000 c. c. of virus solution..	.....do.....	.....do.....	.....do.....	Do.
1 gm. to 2,600 c. c. of virus solution..	.....do.....	.....do.....	.....do.....	Do.
Virus without addition.....	.....do.....	.....do.....	.....do.....	Do.
Tap water only (control).....	.....do.....	.....do.....	.....do.....	All healthy.

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TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

NITRIC ACID—continued

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. to 200 c. c. of virus solution	Sept. 5, 1914	Sept. 9, 1914 (4 days later).	10 Connecticut Broadleaf.	7 plants mosaic.
1 gm. to 300 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	do	7 plants mosaic.
Virus without addition	do	do	do	9 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. to 100 c. c. of virus solution	Jan. 7, 1915	Jan. 8, 1915 (1 day later).	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	7 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	9 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. to 50 c. c. of virus solution	Nov. 30, 1915[a]	Dec. 17, 1915 (17 days later).	do	Do.
1 gm. to 100 c. c. of virus solution	do	do	do	1 plant mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	3 plants mosaic.
1 gm. to 300 c. c. of virus solution	do	do	do	5 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	6 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	8 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	do	7 plants mosaic.
Virus without addition	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

HYDROCHLORIC ACID

1 gm. to 50 c. c. of virus solution	Apr. 25, 1914	Apr. 27, 1914 (2 days later).	10 Connecticut Broadleaf.	All healthy.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	Do.
1 gm. to 800 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	4 plants mosaic.
1 gm. to 2,000 c. c. of virus solution	do	do	do	8 plants mosaic.
1 gm. to 4,000 c. c. of virus solution	do	do	do	9 plants mosaic.
Virus without addition	do	do	do	10 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. to 100 c. c. of virus solution	Jan. 7, 1915	Jan. 8, 1915 (1 day later).	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	6 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	Do.
1 gm. to 800 c. c. of virus solution	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

PHOSPHORIC ACID

1 gm. to 50 c. c. of virus solution	May 25, 1914	May 29, 1914 (4 days later).	10 Maryland Mammoth.	3 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	7 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	Do.
Virus without addition	do	do	do	Do.
Tap water only with weak solution phosphoric acid (control).	do	do	do	All healthy.
1 gm. to 10 c. c. of virus solution	June 9, 1914	June 19, 1914 (10 days later).	do	Do.
1 gm. to 20 c. c. of virus solution	do	do	do	1 plant mosaic.
1 gm. to 50 c. c. of virus solution	do	do	do	3 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	7 plants mosaic.
Virus without addition	do	do	do	10 plants mosaic
Tap water only with weak solution phosphoric acid (control).	do	do	do	All healthy.

[a] Virus filtered clear through filter paper before solutions were made.

TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

PHOSPHORIC ACID—continued

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. to 20 c. c. of virus solution	Nov. 30, 1915[a]	Dec. 18, 1915 (18 days later).	10 Maryland Mammoth.	All healthy.
1 gm. to 50 c. c. of virus solution	do	do	do	Do.
1 gm. to 100 c. c. of virus solution	do	do	do	4 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	8 plants mosaic.
Virus without addition	do	do	do	Do.
Tap water only (control)	do	do	do	All healthy.

CITRIC ACID

Virus saturated with citric acid crystals.	May 23, 1914	May 30, 1914 (7 days later).	10 Maryland Mammoth.	All healthy.
1 gm. to 50 c. c. of virus solution	do	do	do	6 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	4 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	7 plants mosaic.
Virus without addition	do	do	do	9 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. to 10 c. c. of virus solution	June 9, 1914	June 19, 1914 (10 days later).	do	Do.
1 gm. to 20 c. c. of virus solution	do	do	do	2 plants mosaic.
1 gm. to 50 c. c. of virus solution	do	do	do	5 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	7 plants mosaic.
Virus without addition	do	do	do	10 plants mosaic.
Tap water and weak solution citric acid (control).	do	do	do	All healthy.
1 gm. to 20 c. c. of virus solution	Nov. 30, 1915	Dec. 21, 1915 (21 days later).	10 Connecticut Broadleaf.	Do.
1 gm. to 50 c. c. of virus solution	do	do	do	Do.
1 gm. to 100 c. c. of virus solution	do	do	do	1 plant mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	2 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	3 plants mosaic.
Virus without addition	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

ACETIC ACID

1 gm. to 10 c. c. of virus solution	May 25, 1914	June 5, 1914 (11 days later).	10 Maryland Mammoth.	All healthy.
1 gm. to 20 c. c. of virus solution	do	do	do	Do.
1 gm. to 50 c. c. of virus solution	do	do	do	3 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	5 plants mosaic.
1 gm. to 250 c. c. of virus solution	do	do	do	4 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	do	9 plants mosaic.
Virus without addition	do	do	do	Do.
Tap water only (control)	do	do	do	All healthy.
1 gm. to 20 c. c. of virus solution	Nov. 30, 1915	Dec. 18, 1915 (18 days later).	10 Connecticut Broadleaf.	Do.
1 gm. to 50 c. c. of virus solution	do	do	do	Do.
1 gm. to 100 c. c. of virus solution	do	do	do	1 plant mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	5 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	10 plants mosaic.
Virus without addition	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

[a] Virus filtered clear through filter paper before solutions were made.

TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

SODIUM CARBONATE

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. to 50 c. c. of virus solution	Nov. 30, 1915[a]	Dec. 17, 1915 (17 days later).	10 Connecticut Broadleaf.	3 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	4 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	6 plants mosaic.
1 gm. to 300 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	Do.
1 gm. to 500 c. c. of virus solution	do	do	do	8 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	8 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 1,200 c. c. of virus solution	do	do	do	7 plants mosaic.
1 gm. to 1,500 c. c. of virus solution	do	do	do	8 plants mosaic.
Virus without addition	do	do	do	Do.
Tap water only (control)	do	do	do	All healthy.

SODIUM HYDROXID

1 gm. to 50 c. c. of virus solution	Apr. 25, 1914	Apr. 27, 1914 (2 days later).	10 Maryland Mammoth.	All healthy.
1 gm. to 100 c. c. of virus solution	do	do	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	Do.
1 gm. to 800 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	Do.
1 gm. to 2,000 c. c. of virus solution	do	do	do	1 plant mosaic.
1 gm. to 4,000 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 8,000 c. c. of virus solution	do	do	do	Do.
Virus without addition	do	do	do	Do.
Tap water only (control)	do	do	do	All healthy.

MANGANESE SULPHATE

1 gm. to 12.5 c. c. of virus solution	Oct. 13, 1915[a]	Dec. 28, 1915 (76 days later).	10	7 plants mosaic.
1 gm. to 25 c. c. of virus solution	do	do	10	8 plants mosaic.
1 gm. to 50 c. c. of virus solution	do	do	10	9 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	10	10 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	10	Do.
1 gm. to 500 c. c. of virus solution	do	do	10	9 plants mosaic.
Tap water only (control)	do	do	10	All healthy.

SODIUM CHLORID

1 gm. to 25 c. c. of virus solution	Jan. 24, 1916	Mar. 5, 1916 (41 days later).	10	All healthy.
1 gm. to 50 c. c. of virus solution	do	do	10	Do.
1 gm. to 100 c. c. of virus solution	do	do	10	7 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	10	Do.
1 gm. to 400 c. c. of virus solution	do	do	10	5 plants mosaic.
Tap water only (control)	do	do	10	All healthy.

ALUMINIUM SULPHATE

1 gm. to 100 c. c. of virus solution	Feb. 19, 1916	Mar. 7, 1916 (17 days later).	10	3 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	10	10 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	10	8 plants mosaic.
Virus without addition	do	do	10	6 plants mosaic.
Tap water only (control)	do	do	10	All healthy.

[a] Virus filtered clear through filter paper before solutions were made.

TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

LITHIUM NITRATE

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. to 50 c. c. of virus solution	Feb. 19, 1916	Mar. 28, 1916 (19 days later).	10 Connecticut Broadleaf.	6 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	Do.
1 gm. to 600 c. c. of virus solution	do	do	do	Do.
1 gm. to 800 c. c. of virus solution	do	do	do	8 plants mosaic.
Virus without addition	do	do	do	Do.
Tap water only (control)	do	do	do	All healthy.

SODIUM NITRATE

1 gm. to 50 c. c. of virus solution	Feb. 19, 1916	Mar. 28, 1916 (38 days later).	10 Connecticut Broadleaf.	10 plants mosaic.
1 gm. to 100 c. c. of virus solution	do	do	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	Do.
1 gm. to 600 c. c. of virus solution	do	do	do	Do.

LEAD NITRATE

1 gm. to 200 c. c. of virus solution	Feb. 19, 1916	Mar. 28, 1916 (38 days later).	10 Connecticut Broadleaf.	9 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	Do.
1 gm. to 800 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	Do.
Virus without addition (used for sodium nitrate and lead nitrate solutions).	do	do	do	Do.
Tap water only (control) for sodium nitrate and lead nitrate solutions.	do	do	do	All healthy.

SILVER NITRATE

1 gm. to 100 c. c. of virus solution	Nov. 15, 1916[a]	Dec. 18, 1916 (33 days later).	10 Connecticut Broadleaf.	2 plants mosaic.
1 gm. to 300 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,500 c. c. of virus solution	do	do	do	Do.
Virus without addition	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

MERCURIC CHLORID

1 gm. to 100 c. c. of virus solution	Oct. 8, 1915[b]	Nov. 13, 1915 (36 days later).	10	All healthy.
1 gm. to 300 c. c. of virus solution	do	do	10	6 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	10	8 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	10	9 plants mosaic.
1 gm. to 1,500 c. c. of virus solution	do	do	10	10 plants mosaic.
Virus without addition	do	do	10	9 plants mosaic.
Tap water only (control)	do	do	10	All healthy.
1 gm. to 100 c. c. of virus solution	Nov. 16, 1916[a]	Dec. 19, 1916 (33 days later).	10 Connecticut Broadleaf.	4 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	2 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	5 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	6 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,500 c. c. of virus solution	do	do	do	Do.
Pure paper-filtered virus	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

[a] Virus filtered clear through filter paper before solutions were made.
[b] Virus not passed through filter paper before solutions were made.

TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

POTASSIUM PERMANGANATE

Concentration.	Solution prepared.	Inocculation made.	Plants used.	Results.
1 gm. to 100 c. c. of virus solution	Aug. 25, 1915[a]	Aug. 28, 1915 (3 days later).	10	All healthy.
1 gm. to 200 c. c. of virus solution	do	do	10	10 plants mosaic.
1 gm. to 500 c. c. of virus solution	do	do	10	Do.
1 gm. to 800 c. c. of virus solution	do	do	10	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	10	9 plants mosaic.
1 gm. to 1,500 c. c. of virus solution	do	do	10	10 plants mosaic.
Virus without addition	do	do	10	8 plants mosaic.
Tap water only and healthy sap (control).	do	do	10	All healthy.

ZINC CHLORID

1 gm. to 50 c. c. of virus solution	Oct. 12, 1915	Dec. 29, 1915 (78 days later).	10 Connecticut Broadleaf.	All healthy.
1 gm. to 100 c. c. of virus solution	do	do	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. to 100 c. c. of virus solution	Feb. 21, 1916	Mar. 30, 1916 (36 days later).	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	3 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	7 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	6 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	8 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	do	7 plants mosaic.
Virus without addition	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

COPPER SULPHATE

1 gm. to 25 c. c. of virus solution	Apr. 28, 1914	May 7, 1914 (9 days later).	10 Connecticut Broadleaf.	All healthy.
1 gm. to 50 c. c. of virus solution	do	do	do	Do.
1 gm. to 100 c. c. of virus solution	do	do	do	Do.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 500 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	8 plants mosaic.
Virus without addition	do	do	do	Do.
Tap water only and weak solution copper sulphate (control).	do	do	do	All healthy.
1 gm. to 100 c. c. of virus solution	Nov. 17, 1915[b]	Dec. 19, 1915 (32 days later).	do	1 plant mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	5 plants mosaic.
1 gm. to 400 c. c. of virus solution	do	do	do	7 plants mosaic.
1 gm. to 600 c. c. of virus solution	do	do	do	9 plants mosaic.
1 gm. to 800 c. c. of virus solution	do	do	do	10 plants mosaic.
1 gm. to 1,000 c. c. of virus solution	do	do	do	8 plants mosaic.
1 gm. to 1,200 c. c. of virus solution	do	do	do	7 plants mosaic.
1 gm. to 1,500 c. c. of virus solution	do	do	do	10 plants mosaic.
Virus without addition	do	do	do	8 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

CARBOLIC ACID

1 gm. to 100 c. c. of virus solution	Nov. 16, 1915[b]	Dec. 18, 1915 (32 days later).	10 Connecticut	10 plants mosaic.
1 gm. to 200 c. c. of virus solution	do	do	do	Do.
1 gm. to 400 c. c. of virus solution	do	do	do	Do.
1 gm. to 600 c. c. of virus solution	do	do	do	Do.
1 gm. to 800 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,000 c. c. of virus solution	do	do	do	Do.
1 gm. to 1,500 c. c. of virus solution	do	do	do	Do.
Virus without addition	do	do	do	9 plants mosaic.
Tap water only (control)	do	do	do	All healthy.

[a] Virus unfiltered.
[b] Virus filtered clear through filter paper before solutions were made.

TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

CARBOLIC-ACID SOLUTIONS, CARBOLIC-ACID SOLUTIONS CONTAINING 1 PER CENT OF SODIUM CHLORID, AND PURE SODIUM-CHLORID SOLUTIONS

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. of carbolic acid to 100 c. c. of virus solution.	Feb. 21, 1916	Mar. 15, 1916 (23 days later).	10 Connecticut Broadleaf.	9 plants mosaic.
1 gm. of carbolic acid to 200 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 400 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 600 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	10 plants mosaic.
1 gm. of carbolic acid to 800 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 100 c. c. of 1 per cent sodium-chlorid solutions.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 200 c. c. of 1 per cent sodium-chlorid solutions.	.....do.....	.....do.....	.....do.....	7 plants mosaic.
1 gm. of carbolic acid to 400 c. c. of 1 per cent sodium-chlorid solutions.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 600 c. c. of 1 per cent sodium-chlorid solutions.	.....do.....	.....do.....	.....do.....	9 plants mosaic.
1 gm. of carbolic acid to 800 c. c. of 1 per cent sodium-chlorid solutions.	.....do.....	.....do.....	.....do.....	7 plants mosaic.
1 gm. of sodium chlorid to 50 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	5 plants mosaic.
1 gm. of sodium chlorid to 100 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	8 plants mosaic.
1 gm. of sodium chlorid to 400 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	9 plants mosaic.
Virus without addition	.....do.....	.....do.....	.....do.....	8 plants mosaic.
Tap water only (control)	.....do.....	.....do.....	.....do.....	All healthy.

CREOLIN (PEARSON) SOLUTIONS

1 gm. to 50 c. c. of virus solution	Mar. 2, 1916	Mar. 29, 1916 (27 days later).	10 Connecticut Broadleaf.	3 plants mosaic.
1 gm. to 100 c. c. of virus solution	.....do.....	.....do.....	.....do.....	7 plants mosaic.
1 gm. to 200 c. c. of virus solution	.....do.....	.....do.....	.....do.....	10 plants mosaic.
1 gm. to 400 c. c. of virus solution	.....do.....	.....do.....	.....do.....	Do.
Virus without addition	.....do.....	.....do.....	.....do.....	Do.
Tap water only (control)	.....do.....	.....do.....	.....do.....	All healthy.

CRESOL[a] SOLUTIONS

1 gm. to 100 c. c. of virus solution	Mar. 10, 1916	Mar. 29, 1916 (19 days later).	10 Connecticut Broadleaf.	10 plants mosaic.
1 gm. to 200 c. c. of virus solution	.....do.....	.....do.....	.....do.....	9 plants mosaic.
1 gm. to 400 c. c. of virus solution	.....do.....	.....do.....	.....do.....	10 plants mosaic.
1 gm. to 600 c. c. of virus solution	.....do.....	.....do.....	.....do.....	Do.
Tap water only (control)	.....do.....	.....do.....	.....do.....	All healthy.

CARBOLIC-ACID SOLUTIONS AND PHENOCO[b] SOLUTIONS

1 gm. of carbolic acid to 50 c. c. of virus solution.	Feb. 20, 1917[c]	Mar. 7, 1917 (15 days later).	10 Connecticut Broadleaf.	10 plants mosaic.
1 gm. of carbolic acid to 100 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 200 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of carbolic acid to 400 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.
1 gm. of phenoco to 100 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	7 plants mosaic.
1 gm. of phenoco to 200 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	9 plants mosaic.
1 gm. of phenoco to 400 c. c. of virus solution.	.....do.....	.....do.....	.....do.....	Do.

[a] A mixture of the three isomeric cresols.
[b] Phenoco is a commercial disinfectant composed of emulsified phenol homologues and neutral tar oils.
[c] Prepared with fresh, unfiltered virus.

TABLE I.—*Effects of various chemicals on the infectivity of the virus of the mosaic disease of tobacco*—Continued

CARBOLIC-ACID SOLUTIONS AND PHENOCO SOLUTIONS—continued

Concentration.	Solution prepared.	Inoculation made.	Plants used.	Results.
1 gm. of phenoco to 600 c. c. of virus solution.	Feb. 20, 1917	Mar. 7, 1917 (15 days later).	10 Connecticut Broadleaf.	10 plants mosaic.
1 gm. of phenoco to 800 c. c. of virus solution.	do	do	do	Do.
Virus without addition	do	do	do	7 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. of carbolic acid to 50 c. c. of virus solution.	do	Mar. 27, 1917 (35 days later).	do	9 plants mosaic.
1 gm. of carbolic acid to 100 c. c. of virus solution.	do	do	do	10 plants mosaic.
1 gm. of phenoco to 100 c. c. of virus solution.	do	do	do	Do.
1 gm. of phenoco to 200 c. c. of virus solution.	do	do	do	8 plants mosaic.
Virus without addition	do	do	do	10 plants mosaic.
Tap water only (control)	do	do	do	All healthy.
1 gm. of carbolic acid to 50 c. c. of virus solution.	do	Nov. 11, 1917 (264 days later).	do	4 plants mosaic.
1 gm. of carbolic acid to 100 c. c. of virus solution.	do	do	do	7 plants mosaic.
1 gm. of carbolic acid to 200 c. c. of virus solution.	do	do	do	10 plants mosaic.
1 gm. of carbolic acid to 400 c. c. of virus solution.	do	do	do	8 plants mosaic.
1 gm. of phenoco to 100 c. c. of virus solution.	do	do	do	Do.
1 gm. of phenoco to 200 c. c. of virus solution.	do	do	do	9 plants mosaic.
1 gm. of phenoco to 400 c. c. of virus solution.	do	do	do	10 plants mosaic.
1 gm. of phenoco to 600 c. c. of virus solution.	do	do	do	Do.
1 gm. of phenoco to 800 c. c. of virus solution.	do	do	do	9 plants mosaic.
Virus without addition	do	do	do	Do.
Tap water only (control)	do	do	do	All healthy.

ACETONE

Virus in 10 per cent acetone	Oct. 6, 1915	Dec. 22, 1915 (77 days later).	10 Connecticut Broadleaf.	9 plants mosaic.
Virus in 20 per cent acetone	do	do	do	4 plants mosaic.
Virus in 30 per cent acetone	do	do	do	9 plants mosaic.
Virus in 40 per cent acetone	do	do	do	8 plants mosaic.
Virus in 50 per cent acetone	do	do	do	All healthy.
Tap water only (control)	do	do	do	Do.

The cresols are relatively more powerful germicides than carbolic acid. Behring[1] has shown that for certain vegetative forms cresol may have 4 times and creolin 10 times the germicidal power of carbolic acid. The relative differences in the germicidal action of carbolic acid, cresol, and creolin are not apparent in their effects upon the infective principle of the mosaic disease of tobacco under the conditions of the writer's experiments.

In its germicidal action carbolic acid is supposed to act as a molecule, and not by ionization; and the addition of salt greatly increases its germicidal powers. In the time during which it has been allowed to act in the above experiments, there appears to be no appreciable difference

[1] BEHRING, V. BEKÄMPFUNG DER INFECTIONSKRANKHEITEN. . . . 251 p. Leipzig, 1894.

between the carbolic-acid solution and the carbolic-acid and salt solutions in their effects upon the infective principle of the virus.

Phenoco has been shown by Anderson and McClintic [1] to have a phenol coefficient of 15 when tested upon the typhoid bacillus without organic matter, and a coefficient of 9.86 when tested in the presence of organic matter. Although this preparation appears to be a much more powerful germicide than carbolic acid when tested upon the typhoid bacillus, no especially marked differences are shown when tested upon the infective principle of the mosaic disease of tobacco.

The virus of the mosaic disease very quickly loses its power to infect healthy plants when preserved in the higher strengths of ethyl alcohol. In the following tests the expressed sap was filtered through filter paper and mixed with different proportions, by volume, of absolute alcohol. In the process of obtaining the different strengths, the virus at most was diluted but a few times. This slight dilution is without significance since earlier experiments have shown that a dilution of 1,000 times with water does not materially reduce the power to infect. Throughout these tests the plants in each series were kept under observation for a considerable period after the first appearance of symptoms of the disease. There was no noticeable difference, however, in the inoculation period at any time. At present there is no means of knowing whether or not it is possible for the virus of the mosaic disease to retain a certain degree of viability after the power to infect healthy plants has been lost. The results of any germicidal tests are influenced by the concentration of the germicide and the time during which it acts. It is evident that both factors have had more or less influence upon the results obtained for the alcoholic tests presented here. The effect of different concentrations of alcohol upon the infectivity of the virus is given in Table II.

TABLE II.—*Effect of various concentrations of alcohol upon the infectivity of the virus of the mosaic disease*

VIRUS A (PRESERVED IN 25–80 PER CENT ALCOHOL ON FEBRUARY 27, 1914)

Date of inoculation.	Number of plants.	Variety.	Treatment.	Effect.
Apr. 1	10	Connecticut Broadleaf.	Virus A kept in 50 per cent alcohol 33 days.	1 mosaic.
Do	10	do	Untreated virus A kept 33 days.	8 mosaic.
Apr. 2	10	do	Virus A kept in 25 per cent alcohol 34 days.	7 mosaic.
Apr. 3	20	do	Virus A kept in 50 per cent alcohol 35 days.	4 mosaic.
Do	10	do	Virus A kept in 80 per cent alcohol 35 days.	All healthy.
Do	20	do	Untreated virus A kept 35 days.	14 mosaic.

[1] ANDERSON, J. F., and MCCLINTIC, T. B. METHOD OF STANDARDIZING DISINFECTANTS WITH AND WITHOUT ORGANIC MATTER. *In* Pub. Health and Mar. Hosp. Serv. U. S. Hyg. Lab. Bul. 82, p. 1–34. 1912.

TABLE II.—*Effect of various concentrations of alcohol upon the infectivity of the virus of the mosaic disease*—Continued

VIRUS A (PRESERVED IN 25–80 PER CENT ALCOHOL ON FEBRUARY 27, 1914)—contd.

Date of inoculation.	Number of plants.	Variety.	Treatment.	Effect.
Apr. 4	10	Connecticut Broadleaf.	Virus A kept in 50 per cent alcohol 36 days.	1 mosaic.
Do	10	do	Thin paste obtained from dried and ground leaves from which virus A had been expressed, kept 36 days.	5 mosaic.
Do	10	do	Healthy juice and tap water (control).	All healthy.
Do	20	*N. rustica*	Virus A kept in 50 per cent alcohol 36 days.	Do.
Do	10	do	Virus A kept in 80 per cent alcohol 36 days.	Do.
Do	10	do	Virus A, untreated, kept 36 days.	4 mosaic.
Do	10	do	Healthy juice and tap water (control).	All healthy.
Inoculations made 40 or 41 days later:				
Apr. 8	10	Connecticut Broadleaf.	Virus A kept in 25 per cent alcohol 40 days.	5 mosaic.
Do	30	do	Virus A kept in 50 per cent alcohol 40 days.	All healthy.
Do	10	do	Virus A kept in 80 per cent alcohol 40 days.	Do.
Do	20	do	Healthy juice and tap water (control).	Do.
Apr. 9	10	do	Virus A kept in 50 per cent alcohol 41 days.	Do.
Inoculations made 55 days later:				
Apr. 23	10	do	Virus A kept in 25 per cent alcohol 55 days.	1 mosaic.
Do	10	do	Virus A kept in 50 per cent alcohol 55 days.	All healthy.
Do	10	do	Virus A kept in 80 per cent alcohol 55 days.	Do.
Do	10	do	Virus A, untreated, kept 55 days.	3 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.
Inoculations made 56 days later:				
Apr. 24	10	do	Virus A kept in 25 per cent alcohol 56 days.	5 mosaic.
Do	30	do	Virus A kept in 50 per cent alcohol 56 days.	All healthy.
Do	10	do	Virus A kept in 80 per cent alcohol 56 days.	Do.
Do	10	do	Virus A, untreated, kept 56 days.	6 mosaic.
Do	10	do	Healthy juice and tap water.	All healthy.

TABLE II.—*Effect of various concentrations of alcohol upon the infectivity of the virus of the mosaic disease*—Continued

VIRUS A (PRESERVED IN 25–80 PER CENT ALCOHOL ON FEBRUARY 27, 1914)—contd.

Date of inoculation.	Number of plants.	Variety.	Treatment.	Effect.
Inoculations made 68 days later:				
May 6	10	Maryland Mammoth.	Virus A kept in 25 per cent alcohol 68 days.	6 mosaic.
Do	10	do	Virus A, untreated, kept 68 days.	10 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.
Do	10	do	Alcohol and tap-water solution.	Do.
Inoculations made 91 days later:				
May 29	10	do	Virus A kept in 25 per cent alcohol 91 days.	8 mosaic.
Do	10	do	Virus A, untreated, kept 91 days.	10 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.
Inoculations made 199 days later:				
Sept. 14	10	Connecticut Broadleaf.	Virus A kept in 25 per cent alcohol 199 days.	7 mosaic.
Do	10	do	Virus A, untreated, kept 199 days.	9 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.
VIRUS B (PRESERVED IN 95 PER CENT ALCOHOL ON APRIL 8, 1914)				
Apr. 23	10	Connecticut Broadleaf.	Virus B, untreated, kept 15 days.	4 mosaic.
Apr. 24	10	do	Virus B kept in 95 per cent alcohol 16 days.	All healthy.
Do	10	do	Virus B, untreated, kept 16 days.	6 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.
VIRUS C (PRESERVED IN 25–90 PER CENT ALCOHOL ON APRIL 27, 1914)				
May 2	10	Maryland Mammoth.	Virus C kept in 25 per cent alcohol 5 days.	9 mosaic.
Do	10	do	Virus C kept in 30 per cent alcohol 5 days.	7 mosaic.
Do	10	do	Virus C kept in 35 per cent alcohol 5 days.	9 mosaic.
Do	10	do	Virus C kept in 40 per cent alcohol 5 days.	5 mosaic.
Do	10	do	Virus C kept in 45 per cent alcohol 5 days.	6 mosaic.
Do	10	do	Virus C kept in 50 per cent alcohol 5 days.	2 mosaic.
Do	10	do	Virus C kept in 55 per cent alcohol 5 days.	All healthy.
Do	10	do	Virus C kept in 60 per cent alcohol 5 days.	Do.
Do	10	do	Virus C kept in 70 per cent alcohol 5 days.	Do.

TABLE II.—*Effect of various concentrations of alcohol upon the infectivity of the virus of the mosaic disease*—Continued

VIRUS C (PRESERVED IN 25–90 PER CENT ALCOHOL ON APRIL 27, 1914)—continued

Date of inoculation.	Number of plants.	Variety.	Treatment.	Effect.
May 2	10	Maryland Mammoth.	Virus C kept in 80 per cent alcohol 5 days.	All healthy.
Do	10	do	Virus C kept in 90 per cent alcohol 5 days.	Do.
Do	20	do	Virus C, untreated, kept 5 days.	12 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.
Inoculations made 21 days later:				
May 18	10	do	Virus C kept in 25 per cent alcohol 21 days.	9 mosaic.
Do	10	do	Virus C kept in 35 per cent 21 days.	Do.
Do	10	do	Virus C kept in 40 per cent alcohol 21 days.	7 mosaic.
Do	10	do	Virus C kept in 45 per cent alcohol 21 days.	4 mosaic.
Do	10	do	Virus C kept in 50 per cent alcohol 21 days.	All healthy.
Do	10	do	Virus C kept in 55 per cent alcohol 21 days.	Do.
Do	10	do	Virus C untreated, kept 21 days.	10 mosaic.
Do	10	do	Healthy juice and tap water.	All healthy.
Inoculations made 140 days later:				
Sept. 14	10	Connecticut Broadleaf.	Virus C kept in 25 per cent alcohol 140 days.	10 mosaic.
Do	10	do	Virus C kept in 30 per cent alcohol 140 days.	9 mosaic.
Do	10	do	Virus C kept in 35 per cent alcohol 140 days.	10 mosaic.
Do	10	do	Virus C kept in 40 per cent alcohol 140 days.	2 mosaic.
Do	10	do	Virus C kept in 45 per cent alcohol 140 days.	3 mosaic.
Do	10	do	Virus C kept in 50 per cent alcohol 140 days.	All healthy.
Do	10	do	Virus C, untreated, kept 140 days.	8 mosaic.
Do	10	do	Healthy juice, fresh	All healthy.

VIRUS D (PRESERVED IN 80 PER CENT ALCOHOL ON MAY 21, 1914)

May 21	10	Maryland Mammoth.	Virus D kept in 80 per cent alcohol ½ hour.	All healthy.
Do	10	do	Virus D kept in 80 per cent alcohol 1 hour.	Do.
Do	10	do	Virus D kept in 80 per cent alcohol 2 hours.	Do.
Do	10	do	Virus D kept in 80 per cent alcohol 3 hours.	Do.
Do	10	do	Virus D kept in 80 per cent alcohol 4 hours.	Do.

TABLE II.—*Effect of various concentrations of alcohol upon the infectivity of the virus of the mosaic disease*—Continued

VIRUS D (PRESERVED IN 80 PER CENT ALCOHOL ON MAY 21, 1914)—continued

Date of inoculation.	Number of plants.	Variety.	Treatment.	Effect.
May 22	10	Maryland Mammoth.	Virus D kept in 80 per cent alcohol 24 hours.	All healthy.
Do	10	do	Virus D kept in 80 per cent alcohol 28 hours.	Do.
Do	10	do	Virus D untreated	9 mosaic.

VIRUS E (PRESERVED IN 80 PER CENT ALCOHOL ON MAY 27, 1914)

May 27	10	Maryland Mammoth.	Virus E kept in 80 per cent alcohol 2 minutes.	1 mosaic.
Do	10	do	Virus E kept in 80 per cent alcohol 10 minutes.	3 mosaic.
Do	10	do	Virus E kept in 80 per cent alcohol 30 minutes.	1 mosaic.
Do	10	do	Virus E kept in 80 per cent alcohol 60 minutes.	All healthy.
Do	10	do	Virus E untreated	10 mosaic.
Do	10	do	Healthy juice	All healthy.

VIRUS F (PRESERVED IN 25–60 PER CENT ALCOHOL ON MAY 23, 1914)

May 29	10	Maryland Mammoth.	Virus F kept in 25 per cent alcohol 6 days.	7 mosaic.
Do	10	do	Virus F kept in 30 per cent alcohol 6 days.	10 mosaic.
Do	10	do	Virus F kept in 35 per cent alcohol 6 days.	Do.
Do	10	do	Virus F kept in 40 per cent alcohol 6 days.	9 mosaic.
Do	10	do	Virus F kept in 45 per cent alcohol 6 days.	Do.
Do	10	do	Virus F kept in 50 per cent alcohol 6 days.	3 mosaic.
Do	10	do	Virus F kept in 60 per cent alcohol 6 days.	All healthy.
Do	10	do	Virus F, untreated, kept 6 days.	10 mosaic.
Do	10	do	Fresh, healthy juice	All healthy.

VIRUS G (PRESERVED IN 80 PER CENT ALCOHOL ON MAY 31, 1914)

May 31	10	Maryland Mammoth.	Virus G kept in 80 per cent alcohol 2 minutes.	4 mosaic.
Do	10	do	Virus G kept in 80 per cent alcohol 5 minutes.	3 mosaic.
Do	10	do	Virus G untreated	10 mosaic.
Do	10	do	Healthy juice	All healthy.

TABLE II.—*Effect of various concentrations of alcohol upon the infectivity of the virus of the mosaic disease*—Continued

VIRUS H (PRESERVED IN 80 PER CENT ALCOHOL ON MAY 31, 1914)

Date of inoculation.	Number of plants.	Variety.	Treatment.	Effect.
May 31	10	Maryland Mammoth.	Virus H kept in 80 per cent alcohol 2 minutes.	4 mosaic.
Do	10	do	Virus H untreated	10 mosaic.
Do	10	do	Healthy juice and tap water.	All healthy.

VIRUS I (PRESERVED IN 40–75 PER CENT ALCOHOL ON FEBRUARY 12, 1915)

March 3	10	Connecticut Broadleaf.	Virus I kept in 40 per cent alcohol 19 days.	10 mosaic.
Do	10	do	Virus I kept in 50 per cent alcohol 19 days.	5 mosaic.
Do	10	do	Virus I kept in 60 per cent alcohol 19 days.	All healthy.
Do	10	do	Virus I kept in 75 per cent alcohol 19 days.	Do.
Do	10	do	Virus I untreated	6 mosaic.
Do	10	do	Fresh, healthy juice and tap water.	All healthy.

The results of inoculations with alcoholic solutions of virus A show that the virus of mosaic was not affected by 25 per cent alcohol. Its viability, or at least its power to infect, appeared to be unchanged 199 days later. The 50 per cent alcohol solutions were infective 35 to 36 days after preparation, although it is evident that the power to infect had fallen considerably below that of the original virus. Inoculations made with these strengths 40 to 41 days after preparation indicate that the virus in 50 per cent alcohol had become innocuous to healthy plants.

The tests with alcoholic solutions of virus C show very clearly that the virus of mosaic can not long retain its infectious properties in alcohol stronger than 50 to 55 per cent. Five days after preparation all solutions containing 55 per cent or more of alcohol became innocuous. The virus gives evidence of lessening virulence in the 50 per cent alcoholic solution. The power to produce infection appears to have been completely lost by the 50 per cent solution 21 days after preparation. Tests of these solutions 140 days later indicate that the 45 per cent solution was still infectious to healthy plants.

Practically the same results are shown with alcoholic solutions of virus F. All solutions up to 50 per cent were infective 6 days after preparation. As in preceding tests, the 50 per cent solution again gave evidence of decrease in the power to produce mosaic.

Tests with virus D indicate that the virus of the mosaic disease preserved in 80 per cent alcohol became innocuous in less than half an hour.

The virus does not appear to lose instantly its power to produce infection. In a few minutes, however, the virus appears to lose much of its original power to produce infection.

Heintzel [1] treated fresh, mosaic-diseased leaves with 50 per cent alcohol and used the filtered extract for inoculations. This extract produced the disease in healthy plants. In this test it is evident that the water contained in the fresh leaves would reduce the alcohol below the 50 per cent strength used. It also appears that the extract was used at once for inoculation. It would be expected that these extracts would produce infection, since the writer's experiments indicate that the virus may retain its infectivity for a number of days in alcoholic solutions below a 50 per cent strength. Heintzel also treated fresh, unfiltered sap with strong alcohol and filtered out and dried the precipitate. This also produced infection in healthy plants. Since no exact data are given as to the time of treatment and the amount of sap and alcohol used, one can not interpret these results without further details of the methods used.

Koning [2] treated the sap of mosaic-diseased plants with alcohol several times, pouring off the clear, supernatant solution and renewing the alcohol each time. The precipitate which was finally used for inoculation had lost its infectious properties, as would be expected from the writer's results with the higher alcoholic concentrations.

Chloral hydrate in concentrations of 1 in 10, 1 in 20, 1 in 200, 1 in 500, 1 in 800, and 1 in 1,000 parts of virus solution did not appreciably affect the infectivity of the virus after 17 days' treatment.

Tannic acid in concentrations of 1 in 20 and 1 in 50 parts of virus solution killed the infectivity of the virus, as these were no longer infectious after 5 days' treatment. In concentrations of 1 in 100 and 1 in 200 the virus had almost entirely lost its infective properties in the same period. Lecithin had no appreciable effect upon the infectivity of the virus after 5 days in 1, 2, 5, 10, and 20 per cent strengths.

Benzoate of soda in concentrations of 1 in 25, 1 in 50, and 1 in 500 parts of virus solution had apparently not changed the infectivity of the virus after 48 days' treatment. In concentrations of 1 in 100 and 1 in 200 parts of virus solution the virus had not entirely lost its infectivity after 101 days' treatment, although it had been greatly weakened.

Quinine bisulphate in concentrations of 1 in 25, 1 in 500, and 1 in 1,000 parts of virus solution did not noticeably affect the infectivity of the virus after 19 days' treatment.

Sodium taurocholate in 1 and 2 per cent strengths did not destroy the infectivity of the virus after 5 days' treatment. In 5 and 10 per cent strengths the virus had completely lost its infectivity in the same period.

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[1] HEINTZEL, K. G. E. CONTAGIÖSE PFLANZENKRANKHEITEN OHNE MICROBEN, UNTER BESONDERER BERÜCKSICHTIGUNG DER MOSAIKKRANKHEIT DER TABAKSBLÄTTER. 46 p., pl. Erlangen, 1900. (Inaugural Dissertation.)

[2] KONING, C. J. DER TABAK. STUDIEN ÜBER SEINE KULTUR UND BIOLOGIE. 86 p., 15 fig. Amsterdam, Leipzig, 1900.

Saponin in 1, 2, 5, 10, and 15 per cent strengths was tested after three days' treatment. The infectivity of the virus was not entirely lost in any of these strengths, but only one or two cases of the mosaic disease resulted in each test, showing that the virus had been greatly weakened in this period.

Virus treated with naphthalene crystals in excess was highly infectious when tested 16 days later.

Virus treated with camphor in excess was highly infectious when tested six days later.

Virus treated with thymol in excess was highly infectious when tested nine days later.

Antiformin (Eimer & Amend, 1916) in a 1 per cent strength had greatly weakened the virus after three days' treatment, while 5 and 10 per cent strengths had destroyed the infectivity in the same time.

Taka-diastase in excess did not appreciably affect the infectivity of the virus after 17 days' treatment.

Formaldehyde in the concentration of 1 part in 100 parts of virus solution was used for inoculation 10 minutes, 1 hour, and 2 hours after being prepared. The infectivity of the virus was greatly weakened, but not wholly destroyed even after 2 hours' treatment. A concentration of 1 in 100 parts of virus solution had entirely lost its infectivity when tested 10 hours later. In other tests formaldehyde in concentrations of 1 in 200, 1 in 400, 1 in 600, 1 in 800, and 1 in 1,000 parts of virus solution were used for inoculation 18 hours after preparation. The results were as follows:

1 part formaldehyde in 200 parts virus solution	All healthy.
1 part formaldehyde in 400 parts virus solution	2 plants mosaic.
1 part formaldehyde in 600 parts virus solution	3 plants mosaic.
1 part formaldehyde in 800 parts virus solution	7 plants mosaic.
1 part formaldehyde in 1,000 parts virus solution	9 plants mosaic.
Virus untreated	8 plants mosaic.
Tap water only (control)	All healthy.

A 4 per cent formaldehyde solution of virus was prepared and inoculated 10 minutes later and gave three plants mosaic. Inoculations made 20 minutes later showed that all infective properties had been destroyed. The original virus gave 10 plants mosaic, and all controls with tap water remained healthy.

These experiments indicate that the virus of the mosaic disease of tobacco is quickly rendered innocuous in a 4 per cent strength of formaldehyde. This strength has been used by the writer to sterilize pots, which were immersed in the solution from 30 minutes to 1 hour before they were taken out and washed.

Glycerin appears to affect the infectivity of the virus of the mosaic disease of tobacco only very slowly in the lower concentrations. Very strong concentrations appear to weaken its infectivity noticeably in some

instances. Solutions prepared and used for inoculation seven days later gave the following results, 10 Connecticut Broadleaf plants being used in each test:

Virus in 20 per cent glycerin strength	7 plants mosaic.
Virus in 50 per cent glycerin strength	5 plants mosaic.
Virus in 72 per cent glycerin strength	4 plants mosaic.
Virus in 80 per cent glycerin strength	7 plants mosaic.
Virus in 90 per cent glycerin strength	2 plants mosaic.
Virus untreated	9 plants mosaic.
Tap water only (control)	All healthy.

In another test solutions used nine days later gave the following results:

Virus in 10 per cent glycerin strength	5 plants mosaic.
Virus in 25 per cent glycerin strength	2 plants mosaic.
Virus in 50 per cent glycerin strength	4 plants mosaic.
Virus in 75 per cent glycerin strength	All healthy.
Virus in 90 per cent glycerin strength	1 plant mosaic.
Virus untreated	8 plants mosaic.
Tap water only (control)	All healthy.

In the higher concentrations of glycerin—that is, 50, 70, 80, and 90 per cent—the virus may show very weak infectious properties for a long time. In some tests the virus showed very weak infectious properties in 80 and 90 per cent strength glycerin after 35 days' treatment. In other tests, made with different lots of virus, these strengths appeared to kill the infective principle of the virus in much shorter periods.

Koning's [1] experiments indicate that, after long periods of treatment, glycerin destroyed the virus. In one experiment fresh, finely cut portions of mosaic-diseased leaves, which were allowed to stand in glycerin all winter, lost their infectivity.

Heintzel [2] states that glycerin did not affect the infectivity of the virus. His experiments, however, can not be considered final, as he added only a few drops of glycerin to the fresh sap. This author does not state how much sap was used, nor give the time of the treatment. His glycerin solutions appeared to be very dilute, and it would appear that they were used for inoculation at once. As a result, glycerin in his tests did not noticeably affect the infectivity of the virus.

The virus of the mosaic disease of tobacco when mixed with talc, kaolin, or soil frequently loses its infectious properties more quickly than when the virus is merely bottled without the addition of any preservative. In one experiment 25 c. c. of virus were added to about equal volumes of soil, of talc, and of kaolin. After the virus had been added and mixed thoroughly, each lot of material was in the condition of a stiff paste. Forty-three days later each lot of material was thoroughly extracted with 70 c. c. of distilled water, and the solutions were inoculated into lots of 10 plants each. The kaolin and the talc preparations

[1] Koning, C. J. op. cit.

[2] Heintzel, G. E. op. cit.

possessed no infectious properties whatever, while the soil preparation and the original bottled virus were still infectious.

In an additional test 50 c. c. of virus were mixed with 72 gm. of kaolin, U. S. P. Thirty-two days later this material still possessed infectious properties. When tested again, 61 days later, it no longer possessed them.

In a test carried out in March, 1917, 200 gms. of finely screened greenhouse soil were mixed to a stiff paste with 50 c. c. of freshly extracted sap obtained from mosaic plants. One hundred gms. of talc, U. S. P., were mixed with 75 c. c. of fresh virus from the same source, and 100 gm. of finely ground, pure quartz sand were mixed with 25 c. c. of virus. A portion of the original virus was set aside in a bottle. The sand, soil, and talc pastes were kept in glass jars and covered with a sheet of heavy paper tied over the mouth. In November, 231 days later, inoculation tests were made with this virus. The talc and soil preparations appeared to have completely lost their infectious properties, but the sand preparation and the original, bottled virus were highly infectious.

Koning's [1] experiments led him to believe that the soil in some manner destroyed the infective principle of the mosaic disease. Heintzel [2] was also of the opinion that the soil weakened the infectious principle of the disease.

SUMMARY

Nitric and hydrochloric acids had little effect upon the infectivity of the virus, except in concentrations approaching 1 gm. in 50 to 100 c. c. of virus solution. Phosphoric, citric, and acetic acids were without effect, except in concentrations approaching 1 gm. in 20 to 50 c. c. of virus solution.

The virus was more sensitive to the effect of sodium hydroxid than to sodium carbonate.

Manganese sulphate, sodium chlorid, aluminium sulphate, lithium nitrate, sodium nitrate, lead nitrate, and silver nitrate had little effect upon the infectivity of the virus.

Mercuric chlorid affected the virus but little.

Potassium permanganate and zinc chlorid affected the infectivity of the virus only in concentrations stronger than 1 gm. in 100 c. c. of virus solution.

Under certain conditions the copper sulphate has shown itself rather toxic to the infective principle.

Carbolic acid, Creolin, cresol, and Phenoco have affected the infective principle but little under the conditions of the experiments, and there appears to be no appreciable difference in their effects. Phenoco, although having a phenol coefficient of 15 when tested upon the typhoid

[1] Koning, C. J. op. cit.

[2] Heintzel, G. E. op. cit.

bacillus, according to Anderson and and McClintic,[1] does not appear to be stronger than carbolic acid or cresol in its effect upon the infective principle of the mosaic disease of tobacco.

Acetone destroys the infective principle of the mosaic disease much less readily than ethyl alcohol. The infective principle is destroyed rather quickly in alcohol stronger than 50 to 55 per cent. Eighty per cent strengths killed the virus in less than half an hour.

Chloral hydrate in the writer's experiments did not appreciably affect the infectivity of the virus. Benzoate of soda and quinine bisulphate affected the virus but little under the conditions of the experiments. Tannic acid appeared to be somewhat less effective than sodium taurocholate or saponin. Naphthalene crystals, camphor, and thymol had no appreciable effect.

Formaldehyde in a 4 per cent strength destroyed the infective principle very quickly. Glycerin, except in very strong concentrations, affects the virus but little.

When mixed with talc, kaolin, or soil, the virus frequently loses its nfectious properties more quickly than when merely bottled without the addition of any preservative.

[1] Anderson, J. F., and McClintic, T. B. op. cit.

A STUDY OF THE PHYSICAL CHANGES IN FEED RESIDUES WHICH TAKE PLACE IN CATTLE DURING DIGESTION

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INTRODUCTION

The data reported in this paper were obtained by the senior writer and others at the Georgia Agricultural Experiment Station in making studies on the rate of passage of feed residues through the steer and its influence on digestion coefficients.[1] Most of the investigations on digestion in cattle have been made from a chemical rather than from a physical standpoint, yet the importance of the part physics plays has been frequently referred to.[2] This report covers the physical changes which took place in the rations studied during the process of digestion and covers these changes with relation to the several organs and steps in digestion rather than the process of digestion as a whole.

METHOD OF CONDUCTING THE INVESTIGATION

Animals.—The animals used in this work were high-grade 3-year-old Tennessee-bred Shorthorn steers. They had been used in the nutrition studies[3] in the winter of 1915–16, after which they were killed late in the spring of 1916 when slaughter tests were made.

Feeds.—Corn silage and cottonseed meal especially prepared were the feeds used in these experiments. The corn silage was prepared by having the ears pulled off before it went into the silo. While this resulted in a higher percentage of fiber and a lower percentage of nitrogen-free extracts and other nutrients, it had the desired mechanical properties. Silage that would not pass through a 2-mm. screen was wanted. The meal was of the best quality. It was prepared by first passing it through a 15-mesh screen and later through a 20-mesh screen. This removed all except the

[1] Ewing, P. V., and Smith, F. H. A study of the rate of passage of food residues through the steer and its influence on digestion coefficients. *In* Jour. Agr. Research, 10, no. 2, p.55–63. 1917.

[2] Smith, R. M. The physiology of the domestic animals. p. 337. Philadelphia, 1889.

[3] Ewing, P. V., Wells, C. A., and Smith, F. H. The associative digestibility of corn silage and cottonseed meal in steer rations. Pt. 2. Ga. Agr. Exp. Sta. Bul. 125, p. 149–164, 1 fig. 1917.

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most finely ground hulls, the result being a poor grade of cottonseed flour. Even on soaking or after passing through a steer no particles were over 2 mm. in size. The composition of these feeds is given in Table I.

TABLE I.—*Average analyses of feeds used*

Feed.	Dry matter.	Ash.	Nitrogen.	Crude fiber.	Nitrogen-free extract.	Fat.
Silage	25.65	1.62	0.157	9.82	12.80	0.43
Cottonseed meal	90.12	5.57	6.220	5.70	32.24	7.73

RATIONS.—The composition of the rations fed prior to making the slaughter tests is given in Table II. These particular rations were used because of the bearing they had on the previously conducted digestion experiments. In regard to the rations used it should be noted from the table that the rations were so planned that of the two actual rations fed—that is, those rations in which both feeds appeared—we have actual qualitative and quantative slaughter tests of the component parts of the rations.

WEIGHING AND SAMPLING OF FEEDS.—A sample of the silage was taken from each feed and placed in an air-tight specimen jar, to which a sufficient amount of toluene was added to preserve properly the sample. This was kept in a refrigerator at a temperature of about 4° to 6° C., after which it was weighed, air-dried, reweighed, ground, and sampled for chemical analysis. Samples of the cottonseed meal were collected during the feeding period and analyzed at the close of the test.

FEEDING PERIOD.—In every case the minimum feeding period prior to slaughter was 18 days, which had been found from previous work to be sufficient to clear satisfactorily the alimentary tract of residues from previous feeds.

SLAUGHTER TESTS.—The slaughter tests upon which this work is based were made with six steers, as shown in Table II, which gives the numbers, dates of slaughter, rations fed, and other data. Prior to slaughter these steers had been fed regularly night and morning on the test rations. They were killed, by shooting, at 12.30 p. m., 30 minutes after consuming a half-feed, at which time they were considered as approximating the nearest normal condition, so far as the contents of the entire alimentary tract and the several organs were concerned. The esophagus was then exposed and tied, as was the rectum. As rapidly as possible the several organs were then secured so as to prevent passing of residues from one organ to another. Afterwards the contents of each organ were weighed, dried, and sampled; physical analyses were then made separately (Table II).

TABLE II.—*Summary of tests made in 1916*

Steer No.	Date.	Weight of steer.	Percentage composition of ration.		Daily feeds.		Average daily dry matter in—		Average time in passage through steer.
			Silage.	Cotton-seed meal.	Silage.	Cotton-seed meal.	Feeds.	Feces.	
		Kgm.			*Kgm.*	*Kgm.*	*Kgm.*	*Kgm.*	*Hours.*
52.....	May 31	385	40	0	6.848	0.000	1.6565	0.8206	125.8
49.....	June 3	380	60	0	10.270	.000	2.6340	1.2446	90.0
46.....	June 8	362	40	60	6.848	2.016	3.4730	1.5022	80.6
45.....	June 11	385	60	40	10.270	1.338	3.8400	1.5186	74.0
44.....	April 27	395	0	60	.000	2.016	1.8170	.5231	74.0
53.....	May 29	358	0	40	.000	1.338	1.2060	.3450	70.0

METHOD OF MAKING PHYSICAL ANALYSIS.—A quantity of each lot of residues of which an analysis was to be made was placed in a 1,200-c. c. shaking flask, after which distilled water was added to make up to 400 c. c. This was then shaken in a machine operated by motor for two hours at room temperature. The contents were placed on a brass screen with 2-mm. round holes; the mixing flask was cleansed, and the residue washed on the screen with 100 c. c. of distilled water.[1] The residue was removed from the screen to a mortar containing 50 c. c. of water and was ground very lightly; after this it was placed on the screen, 50 c. c. of water being used to clean the mortar. The whole was washed again with 100 c. c. of water, the process being repeated until the residue was apparently free from true feces or the finely divided particles. The corn-silage residue, the part left on the screen and designated as fraction "a" was then transferred to filter paper, dried, and weighed.

After fraction a had been removed, the filtrate washings were treated with 3 gms. of potassium aluminium sulphate, [$K_2SO_4.Al_2(SO_4)_3.24\ H_2O$], placed in a mixing flask, made up to 1,000 c. c. with distilled water, shaken at room temperature for one hour, and allowed to stand a few minutes to permit partial sedimentation. It was then filtered by decantation and washed with distilled water until free from sulphates. The residue constituted fraction b. The filtrate with washings constituted fraction c, which for this study was not considered. After the separation had been completed, fractions a and b were dried in an oven at 105° C. to constant weight.

DISCUSSION OF RESULTS

The data presented in Table III provide material for making studies on the physical changes in feed that take place during the digestion of coarse feeds in cattle. This is especially true of the first two tests, in which silage alone was fed, to a lesser extent in case of the next two rations containing both silage and cottonseed meal, while for certain phases of this study the last two rations can not be considered, since the

[1] Distilled water was used for all purposes in making the separation.

feeds used were already finely divided and required no comminution. All the rations and tests give information on the time food remains in each organ.

TABLE III.—*Physical changes that occur during the digestion of coarse feeds in the stomach and intestines of cattle*

Steer No.	Gross weight of contents.	Percentage organ content is of body content.	Weight of dry matter in contents of organs.		Percentage of dry matter in contents of organs.		Percentage dry-matter content of organs is of total dry-matter contents.	Percentage dry-matter content is of organ contents.
			Above 2 mm.	Below 2 mm.	Above 2 mm.	Below 2 mm.		
	Kgm.		*Kgm.*	*Kgm.*				
RUMEN AND RETICULUM								
52	49.124	73.31	1.754	2.748	38.9	61.1	69.4	9.2
49	51.150	72.31	2.471	2.431	50.5	49.5	67.5	9.7
46	46.255	71.48	2.369	3.153	42.9	57.1	66.1	11.9
45	47.000	71.80	3.000	2.265	56.9	43.1	62.9	11.2
44	41.799	69.16	.000	2.171	.0	100.0	59.2	5.2
53	24.454	66.85	.000	1.326	.0	100.0	58.5	5.4
OMASUM								
52	7.460	11.14	0.352	0.779	31.1	68.9	17.5	15.2
49	8.140	11.49	.451	.803	36.0	64.0	17.3	15.4
46	3.775	5.83	.190	.613	23.7	76.3	9.6	21.1
45	4.900	7.48	.210	.757	21.7	78.3	11.5	19.8
44	4.890	8.10	.000	.467	.0	100.0	12.7	9.5
53	3.000	8.20	.000	.284	.0	100.0	12.5	8.3
ABOMASUM								
52	1.051	1.57	0.038	0.105	26.3	73.7	2.2	13.6
49	1.690	2.38	.067	.166	28.7	71.3	3.2	13.8
46	2.630	4.07	.120	.450	21.0	79.0	6.8	21.7
45	2.260	3.45	.080	.325	19.7	80.3	4.8	17.9
44	2.510	4.15	.000	.205	.0	100.0	5.6	8.2
53	2.019	5.52	.000	.156	.0	100.0	6.9	7.7
SMALL INTESTINE								
52	3.896	5.81	0.028	0.218	10.1	89.9	3.7	6.2
49	3.976	5.62	.044	.279	13.6	86.4	4.4	8.1
46	6.060	9.36	.030	.570	5.0	95.0	7.2	9.9
45	6.000	9.17	.053	.420	11.2	88.8	11.3	15.8
44	6.192	10.25	.000	.401	.0	100.0	10.9	6.5
53	3.100	8.47	.000	.196	.0	100.0	8.6	6.3
LARGE INTESTINE								
52	5.469	8.17	0.079	0.392	16.7	83.3	7.2	8.6
49	5.804	8.20	.102	.446	18.6	81.4	7.6	9.4
46	5.594	9.25	.225	.640	26.0	74.0	10.3	14.4
45	5.300	8.10	.145	.650	18.2	81.8	9.5	15.0
44	5.033	8.34	.000	.426	.0	100.0	11.6	8.5
53	4.008	10.96	.000	.307	.0	100.0	13.5	7.7

COMMINUTION

In but very few instances have efforts been made to measure the extent, efficiency, and results of the comminution of coarse feeds during the process of digestion. It has been recognized at all times that the comminution of feeds was important in order to obtain the most complete digestion. For example, old horses and cattle fail to fatten or secure the fullest benefits from coarse feeds consumed when their teeth become worn out or unsound, mastication being the most important step in the comminution of feed, as shown by our results. The extent of disintegration of coarse feeds is dependent on several factors, but from our figures it is evident that the comminution of silage is over 90 per cent efficient, with 2 mm. as the dividing line. The silage as fed was 100 per cent over 2 mm., while the residue of the feces over 2 mm. was in every case less than 20 per cent. Since the dry matter of the feces did not amount to 50 per cent of the dry matter of the feeds, it is quite apparent that over 90 per cent of the silage consumed was reduced from above 2 mm. to below 2 mm.

The data showing the amounts of the contents of the organs above and below 2 mm. furnish an indication of the extent of comminution taking place in the various organs. The extent of comminution is determined by difference, a method which possesses certain inaccuracies, but which is probably the best method applicable to our figures.

COMMINUTION IN MOUTH, RUMEN, AND RETICULUM

A small amount of comminution may take place during prehension. Most of it takes place, however, as a result of mastication, which may be of one or two kinds: The preliminary, to prepare the bolus for deglutition, or the final, which takes place much more slowly and completely when the animal ruminates.[1] In this study we can secure a measure of the total extent of comminution accomplished by mastication and rumination by knowing the condition of the food as it is fed the animal and by measuring the physical condition of the contents of the rumen, reticulum, and other organs.

A study of the data given in Tables IV and V shows that in the first two rations, which were made up of silage alone, the extent of comminution taking place before the food leaves the rumen and reticulum amounted to 65.8 per cent in the smaller ration and to 58.5 per cent in the larger ration. This is in accord with previous results,[2] when it was found that a smaller quantity of silage was comminuted to a greater extent than a larger quantity. When to these two rations were added the 60 and 40 per cent of cottonseed meal, an increase resulted in the extent of comminution of 5.1 and 10.5 per cent.

[1] SMITH, FREDERICK. MANUAL OF VETERINARY PHYSIOLOGY. Ed. 4, p. 157. London, 1912.
[2] EWING, P. V., WELLS, C. A., and SMITH, F. H. Op. cit.

TABLE IV.—*Extent of comminution in early stages of digestion and effect of absorption in later stages*

[Figures represent changes effected in reducing proportion of feed residues from above 2 mm. to below 2 mm.]

Stage of digestion.	Ration.			
	1	2	3	4
Mastication and one-half rumen and reticulum	61.1	49.5	57.1	43.1
One-half rumen and reticulum / One-half omasum	7.8	14.5	19.2	35.2
One-half omasum / One-half abomasum	4.8	7.3	2.7	2.0
One-half abomasum / One-half small intestines	16.2	15.1	16.0	8.5
One-half small intestines / One-half large intestines	−6.6	−5.0	−21.0	−7.0
One-half large intestines	0.7	7.0	−6.8	−1.7

TABLE V.—*Calculated amounts of comminution taking place in the several organs during digestion*

Organ.	Ration.			
	1	2	3	4
Rumen and reticulum (including mastication)	65.8	58.5	70.9	69.0
Omasum	6.3	10.9	10.9	18.6
Abomasum	10.5	11.2	9.4	5.3
Small intestines	4.8	5.1	−2.5	.8
Large intestines	−2.6	4.5	−17.3	−5.2

In making these observations on the extent of comminution in the rumen it should be noted that a portion of the last half-feed had undoubtedly not been ruminated. This discrepancy would not apply beyond the rumen and recticulum to an appreciable extent. The extent of comminution that takes place in the rumen and reticulum is in reverse order to the extent of comminution as a result of mastication. The most complete mastication occurred with ration 1, which was made up of a small amount of silage, while it is with this ration that the least comminution in the rumen and reticulum is indicated. The largest amount of comminution in the rumen and reticulum is in ration 4.

COMMINUTION IN OMASUM

In general, the amount of comminution that takes place in the omasum is rather constant, ranging from 6.3 to 18.6 per cent, the greater amount taking place with those rations made up of silage and cottonseed meal, and the least amount in the ration containing the smaller amount of silage alone.

COMMINUTION IN ABOMASUM

A more constant amount of comminution takes place in the abomasum than in the omasum, and the rations associated with the greater comminution are those made up of a higher percentage of coarse feeds, as in the silage alone.

COMMINUTION IN SMALL AND LARGE INTESTINES

Owing to the nature of digestion from a physical standpoint, the early part of the digestion period is consumed largely with a process of comminution and preparation, and comparatively little absorption takes place. During the later stages of digestion the extent of comminution is presumably much less, and absorption is much greater. The existence of this condition is brought out clearly in Tables IV and V, where it is seen that the extent of any comminution that takes place in the intestines is either partially or completely overshadowed by absorption. Since the absorption takes place much more rapidly with those particles under 2 mm. than with those over 2 mm., we obtain several negative percentage comminutions. The fact that the figures obtained for the intestines are composite figures representing two opposite effects, comminution and absorption, renders them of but little value in making deductions, since no method is available which will enable us to determine the extent of either factor alone.

RELATION BETWEEN TIME FOOD REMAINS IN ORGANS AND EXTENT OF COMMINUTION

A study of Tables IV to VI shows a relationship between the extent of comminution, the time the food residues remain in the organs, and the functional activities of the several organs. In general, the food residues remain the shortest time in the most active organs and the longest time in the most inactive. This is seen in the case of the abomasum, probably the most active organ functionally, in which food remains on the average only 2.83 hours, while in the rumen and reticulum, probably the most inactive functionally of all the organs, in which the food remains on the average over 60 hours, we find the extent of comminution not to be in proportion to the time. From a study of the figures it is seen that the extent of comminution is a resultant of two forces, time and functional activity, the functional activity being the stronger of the two influences. Thus, even though the food mass remain in the omasum for approximately three times as long as in the abomasum, the extent of comminution is about the same in both organs, the greater functional activity of the abomasum replacing the time factor in the omasum. As previously noted, the extent of comminution in the intestines is confused by absorption, so that no studies can be made on these data with reference to these relationships.

TABLE VI.—*Time required for the passage of residues through the several organs*

Organ.	Period of passage with ration No. —						
	1	2	3	4	5	6	Average.
	Hours.	*Hours.*	*Hours.*	*Hours.*	*Hours.*	*Hours.*	*Hours.*
Rumen and reticulum	92.2	65.0	57.6	53.0	51.8	46.8	61.07
Omasum	14.0	11.4	4.7	5.5	6.0	5.7	7.88
Abomasum	2.0	2.1	3.3	2.6	3.1	3.9	2.83
Small intestines	7.3	5.2	7.5	6.8	7.6	5.9	6.72
Large intestines	10.3	7.3	8.0	6.0	6.2	7.7	7.58

To have made this study more complete would have required accurate measurements of the capacities of the several organs. However, the dry-matter contents of the several organs are probably the most satisfactory for making these studies. Some linear measurements of the several organs were made, and these are presented in Table VII.

TABLE VII.—*Linear measurements of organs*

Steer No.	Esophagus.	Abomasum.	Small intestines.	Large intestines.	Cæcum.[a]
	Meters.	*Meters.*	*Meters.*	*Meters.*	*Meters.*
52	1.05	0.74	41.00	10.00	0.55
49	1.10	.75	39.50	9.80	.58
46	1.00	.68	37.10	10.58	.44
45	1.00	.69	39.70	8.72	.53
44	1.06	.76	40.00	10.21	.65
53	.95	.71	36.75	7.02	.38

[a] Also figured in large intestines.

CONCLUSIONS

(1) More than half of the comminution that takes place in average rations containing coarse feeds takes place as a result of mastication.

(2) When rations are incompletely masticated, a higher percentage of the comminution takes place in the rumen and reticulum.

(3) About the same extent of comminution takes place in the omasum and abomasum.

(4) The amount of comminution is not alone dependent on the time food residues remain in the several organs, but it is also dependent on the functional activity of the several organs.

(5) Absorption takes place to the greatest extent in the intestines.

(6) Absorption tends greatly to lessen the quantity of residues below 2 mm. and proportionately to increase that above 2 mm.

(7) The comminution of silage-alone rations during the process of digestion is over 90 per cent efficient, with 2 mm. as the dividing line.

SUNSCALD OF BEANS[1]

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INTRODUCTION

The seed-bean industry is new in northern Colorado. In the Greeley district eastern seed houses contracted for 3,000 acres in 1916; 10,000 in 1917; and it is estimated that 25,000 acres will be contracted for in 1918. Forty thousand acres in Weld County were in beans (*Phaseolus vulgaris*), both seed and commercial, in 1917, the acreage having been distributed among 27 varieties. Yields of 35 to 45 bushels per acre indicate that irrigation and climatic conditions are favorable to bean production. Anthracnose is rare; rust appears only where rotation has been neglected; bacterial-blight is yet uncommon.

Late in 1916, before the beans had been harvested, a spotting and streaking[2] of bean pods generally was observed. In all stages it had the appearance of a bacterial infection. Microscopic examination was made of the traumatic tissue; but no bacteria, or suggestion of bacteria, were found. Cultures were made on beef agar, potato agar, bean agar, string beans, and in beef bouillon; but no organism developed, other than an occasional contamination.

THE DISEASE

In northern Colorado beans are planted about May 20. By planting 60 pounds of seed to the acre in rows 28 inches apart a heavy stand of vine is obtained. After three irrigations the pods are well filled and approaching maturity. Usually no water is applied after the middle of August. From this time on, the leaves gradually desiccate and curl exposing the ripening pods beneath. Then the spotting appears. At this period the green stage has passed, and the pods are whitening.

The first indications of disease are very tiny brown or reddish spots upon the upper or outer valve away from the center of the plant. These spots gradually lengthen until they appear as short streaks running backward and downward from the ventral toward the dorsal suture. In two days the spots have increased to areas of brown water-soaked tissue, some-

[1] This problem was originally taken up as a joint problem with Prof. W. G. Sackett, Bacteriologist of the Colorado Agricultural Experiment Station, but his absence from the State in the fall of 1917 made another arrangement necessary. However, the writer is indebted to Prof. Sackett for the results of some microscopical and cultural study.

[2] This "streak" was first mentioned by (Sackett SACKETT, W. G. DISEASES OF BEANS. *In* Colo. Agr. Exp. Sta. Bul. 226, p. 21–31, 6 fig. 1917.)

times slightly sunken. If the spread has been rapid, the color is a good brown, sometimes tinged with red, extending over a majority of the exposed surface, and sometimes over all of it. On some varieties the entire exposed surface does not become covered, but spots 3 to 4 mm. in diameter grow to be the largest, while new spots are constantly appearing. Often small spots coalesce into larger ones, giving them an irregular shape. Eventually this spotting may appear on the underside of the pod, but always in lesser quantity. From these spots no organism has been cultured or observed.

EXPERIMENTAL WORK

From August 15 to September 15, 1917, the average maximum temperature was 27.2° C. at the experiment station at Greeley. No extremes of weather were experienced. There were four partly clouded days; rain fell three times in small amount. At this altitude (4,700 feet) the days during this period were warm and clear, and the heat of the sun seemed very intense.

In those varieties which early showed the lack of water the disease was apparent by August 20. On September 1 some beans of the Green Bountiful variety showed spotting. Plate 64, A, shows six bean pods which were exposed to the sun naturally on the plant and had become well spotted. Plate 64, B, shows the reverse side of the same six pods, but only faint indications of spotting appear in slight amount.

On September 6 experiments were begun in a field of the Bountiful variety.[1] A small table was constructed of four stakes and a muslin sack so placed that one edge came close to a number of bean pods. Pods showing no spotting, and still naturally attached to the plant, were laid on top of the sack, exposed to the sun. Other pods from the same stalk were slipped through a slit made in the edge of the sack in such a manner that the lower sheet of the sack supported the pod in approximately the same position as the exposed pod, while the upper sheet covered it. This gave each pod practically the same conditions and position, except that one was covered with a single covering of thin muslin. All shading leaves were removed, and the table was exposed to the full strength of the sun's rays. Early in the morning the muslin was wet with dew, and any spores or bacteria which might have infected the exposed bean pods had an equal or better chance for contact with the covered one.

Spotting quickly appeared on the exposed pods, while none appeared on the covered ones. On September 13 the experiment was discontinued, and a photograph taken (Pl. 65, A). The pod which went through the experimental period covered is shown at the right, removed from the sack; there is complete absence of spotting. The pod at the left was exposed in the position shown, and is characteristically spotted. The results of seven trials were identical in every way.

[1] The Bountiful variety should not be confused with the Green Bountiful variety used above.

On September 6, bean pods which showed very slight spotting and some showing none at all were tied in muslin sacks and left until September 13. The leaves were removed and no shade covered the plants at any time of the day. Four groups of these pods are shown in Plate 65, B, and are as free from spotting as when placed in the sacks. The two pods at the left show slight traces, which was the condition on September 6.

On September 8 the original experiment was repeated on pods of the Refugee Wax variety. The tables were constructed of stakes and muslin sacks; and pods free from spotting were exposed and inserted in the sacks. The experiment was discontinued on September 16, with the same result as in the previous case. Plate 66, A, shows the results of one of these trials. The pods were placed on a mirror for photographing. On the right is one pod which was half-inserted in the sack, the lower half showing no spotting, the upper half being heavily spotted. The pods at the left were exposed on the table and are heavily spotted on the upper surface; but the lower surface, as revealed in the image, shows no spotting. The two pods at the center were not included in the experiment, but hung naturally on the plant, partially shaded.

In a field of the Hardy Wax variety, in which the leaves were yet green, owing to late irrigation, plants were found which had been crowded over by the wind. Exposed pods were spotted. On the stalks and branches also, long, brown streaks were observed which appeared to be due to the same cause. These streaks were only on the side exposed to the sun. A plant of this type is illustrated in Plate 66, B.

CAUSE OF THE DISEASE

The spotting and streaking of the pods and the streaking of the stems and branches is due to sunscald. When the pods are shaded, as in leafy varieties, little or no spotting occurs. The tissue is bacteriologically sterile.

ECONOMIC IMPORTANCE

No loss is caused by sunscald. Bean pods have been found which were so severely scalded that the seed coats within were slightly stained, but no ill effects were to be observed. Seed saved from pods severely scalded in 1916 and planted in 1917 were normal in every respect. There was no decrease in yield, nor lack of vigor. The pods filled normally, and scalding occurred again as it had done before.

The danger is that the sunscald may be mistaken for bacterial-blight, or that bacterial-blight may be disregarded for scald. Bean pods examined at several places in the East were spotted very finely by spots closely resembling the ones described above. They were diagnosed by pathologists as bacterial-blight spots, because they were so common and had the characteristics of incipient blight infection. Spots of some few examined were not bacterial, and were believed to be slight touches of sunscald.

CONCLUSION

The spotting and streaking of bean pods and stems, herein described is due to sunscald.

No damaging effects due to this scalding have been observed.

The scalding is diminished or prevented by the shade of leaves, but when these wither, it may occur in a period of six days.

No varieties of beans have been observed to be immune from sunscald where sufficiently exposed.

At certain stages of the disease the appearance may easily be mistaken for bacterial infection, and can not be differentiated except upon examination under the microscope.

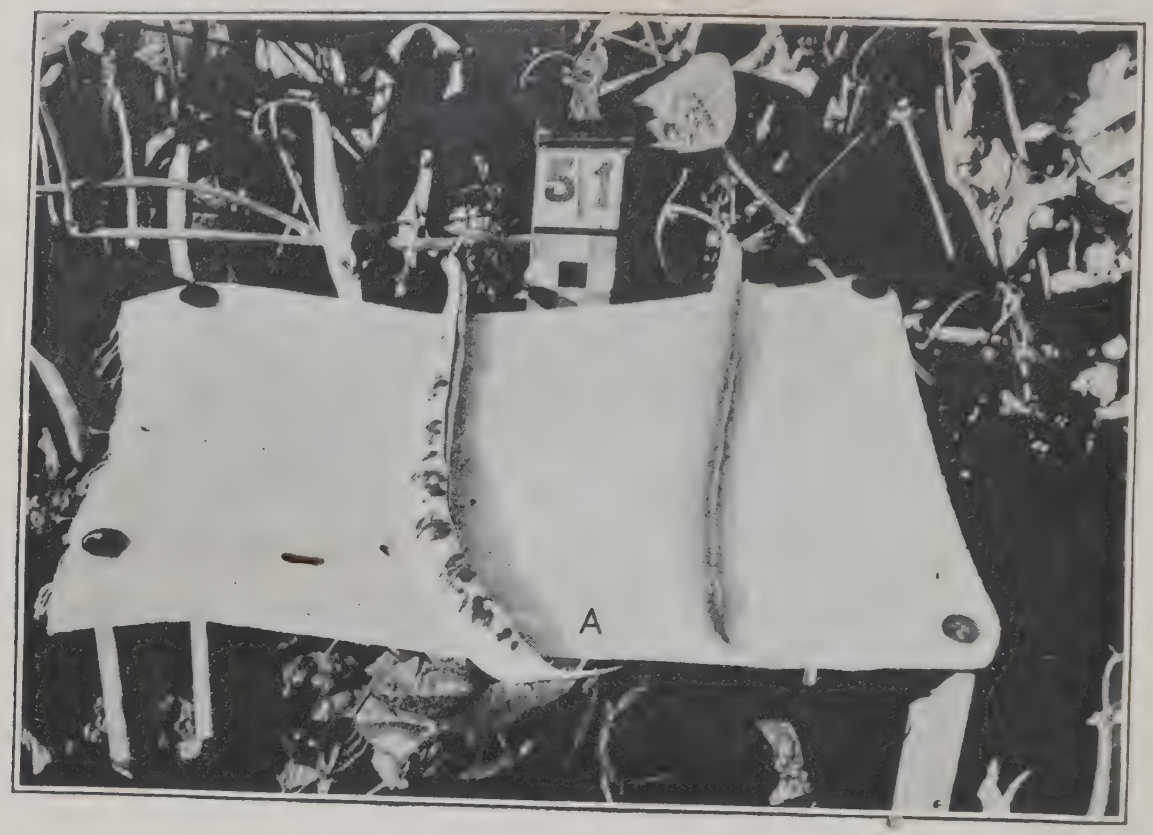
PLATE 64

A.—Six pods of the Green Bountiful variety of beans which showed natural sunscald on September 1.

B.—Reverse side of the six pods shown in A.

A

B


51
A

B

PLATE 65

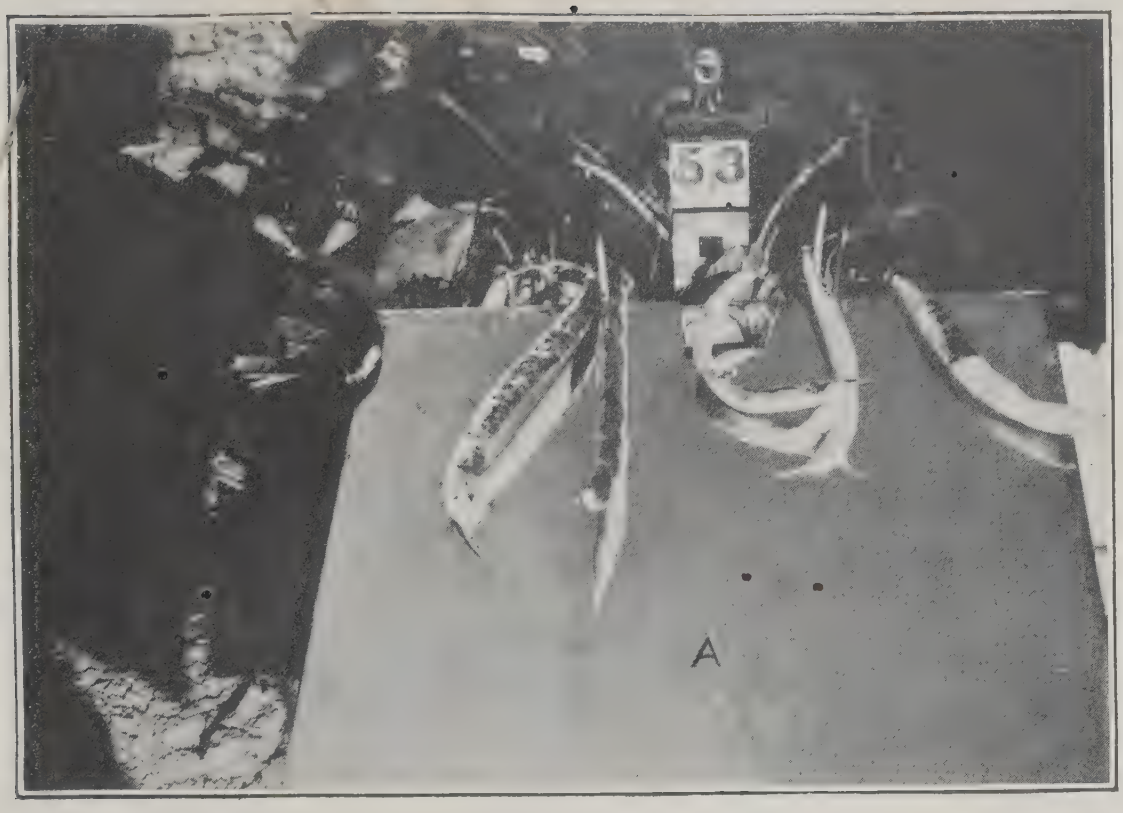
A.—Two pods of beans, the one at the left injured by sunscald, the one at the right having been protected from the rays of the sun. The pod at the left was exposed to the sun in the position shown. The pod at the right was covered by slipping it through a slit on the back edge of the muslin sack.

B.—Four groups of bean pods exposed tied in muslin sacks. The two at the left were slightly spotted as shown when the experiment began.

PLATE 66

A.—Refugee wax beans. The pod at the right was exposed for one-half its length. The image in the mirror shows the freedom from spotting of the underside of the exposed pods.

B.—Hardy wax beans, showing sunscald on the stems, branches, and pods.


53
A

B

A THIRD BIOLOGIC FORM OF PUCCINIA GRAMINIS ON WHEAT[1]

[PRELIMINARY PAPER

By M. N. LEVINE, *Field Assistant, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*, and E. C. STAKMAN, *Head of the Section of Plant Pathology, Department of Agriculture, University of Minnesota*

COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE[2]

Two biologic forms of stemrust have been known to occur on wheat. *Puccinia graminis tritici* Erikss. and Henn. was the only one recognized until recently, when *P. graminis tritici-compacti* Stak. and Piem. was discovered.[3]

Stemrust collected on clumps of volunteer wheat at Stillwater, Okla., October 18, 1917, was found to be different parasitically from both *P. graminis tritici* and *P. graminis tritici-compacti*. Immediately after collection it was sent to University Farm, St. Paul, Minn., where inoculations were begun to determine its identity. It was cultured for several generations on Brown Gloria club wheat, Haynes bluestem (Minn. 169), and Manchuria barley (Minn. 105), during which time its identity was in doubt. Spore measurements made during the same time appeared to indicate that the rust was neither *P. graminis tritici* nor *P. graminis tritici-compacti*.

The discovery of *P. graminis tritici-compacti* and subsequent work with it showed clearly the value of using differential hosts to distinguish between different biologic forms. It has been shown previously[4] that two or more biologic forms could infect many grasses and some cereals equally well but that their action on at least one of the common cereals—wheat, barley, oats, and rye—was sufficiently different to make their determination simple. For instance, *P. graminis tritici* and *P. graminis secalis* attack many grasses and barley equally well; oats are almost immune from both; but the *tritici* form attacks rye only weakly and attacks common wheats heavily, while the *secalis* form attacks rye heavily and

[1] The writers are under obligation to Dr. Charles Drechsler, Field Assistant, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, for participating in the collection of the original material: to Mr. G. R. Hoerner, Assistant in Plant Pathology, Minnesota Agricultural Experiment Station, for the preliminary inoculations: and to Mr. J. G. Leach, Shevlin Fellow, University of Minnesota, for valuable suggestions and for many of the data on the differential hosts for *P. graminis tritici* and *P. graminis tritici-compacti*.

[2] Published, with the approval of the Director, as Paper 121 of the Journal series of the Minnesota Agricultural Experiment Station.

[3] STAKMAN, E. C., and PIEMEISEL, F. J. A NEW STRAIN OF PUCCINIA GRAMINIS. *In* Phytopathology, v. 7, no. 1, p. 73. 1917.

[4] STAKMAN, E. C., and PIEMEISEL, F. J. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON CEREALS AND GRASSES. *In* Jour. Agr. Research, v. 10, no. 9, p. 429–496, pl. 53–59, 1917. Literature cited, p. 493–495.

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can not infect wheat. Rye and wheat are, therefore, differential hosts for the two forms. But the *tritici* and the *tritici-compacti* forms can not be distinguished from each other by their action on grasses, soft wheats, oats, barley or rye. The differential hosts for these two forms are less widely separated taxonomically. It is only by their action on a limited number of different varieties of wheat that they can be distinguished; but the differences on the varieties which do serve as differential hosts are extremely sharp and very consistent.

In order to definitely establish the identity of the rust in question, it was further cultured on the following differential hosts for *P. graminis tritici* and *P. graminis tritici-compacti* (in addition to those mentioned above): Kanred P762 (Kans. 2401), P1066 (Kans. 2415), P1068 (Kans. 2414), Barletta (Minn. 1178), Marquis (Minn. 1239), and Royalton (Minn. 1037)—all *vulgare* wheats.

Club wheat has been found by Stakman and Piemeisel [1] to be highly susceptible to both *P. graminis tritici* and *P. graminis tritici-compacti*, while Haynes bluestem was found very susceptible to the *tritici* form and highly resistant to *tritici-compacti*. The Kansas varieties, P762, P1066, and P1068, have been described by Melchers and Parker [2] as decidedly resistant to *P. graminis tritici compacti* under greenhouse conditions. Barletta, Marquis, and Royalton, on the other hand, are extremely resistant to *P. graminis tritici-compacti*. Inoculation experiments proved all of these varieties to be very susceptible to the rust found in Oklahoma. The results of the inoculations, which extended over a period of six months, during which 10 successive transfers were made, are given in diagram 1.

This diagram shows that all the wheat varieties tested were susceptible to the new rust. These varieties, as already mentioned, are differential hosts for *P. graminis tritici* and *P. graminis tritici-compacti* (Table I). By using these differential hosts it was possible to ascertain that the new rust was not a mixture of *P. graminis tritici* and *P. graminis tritici-compacti*.

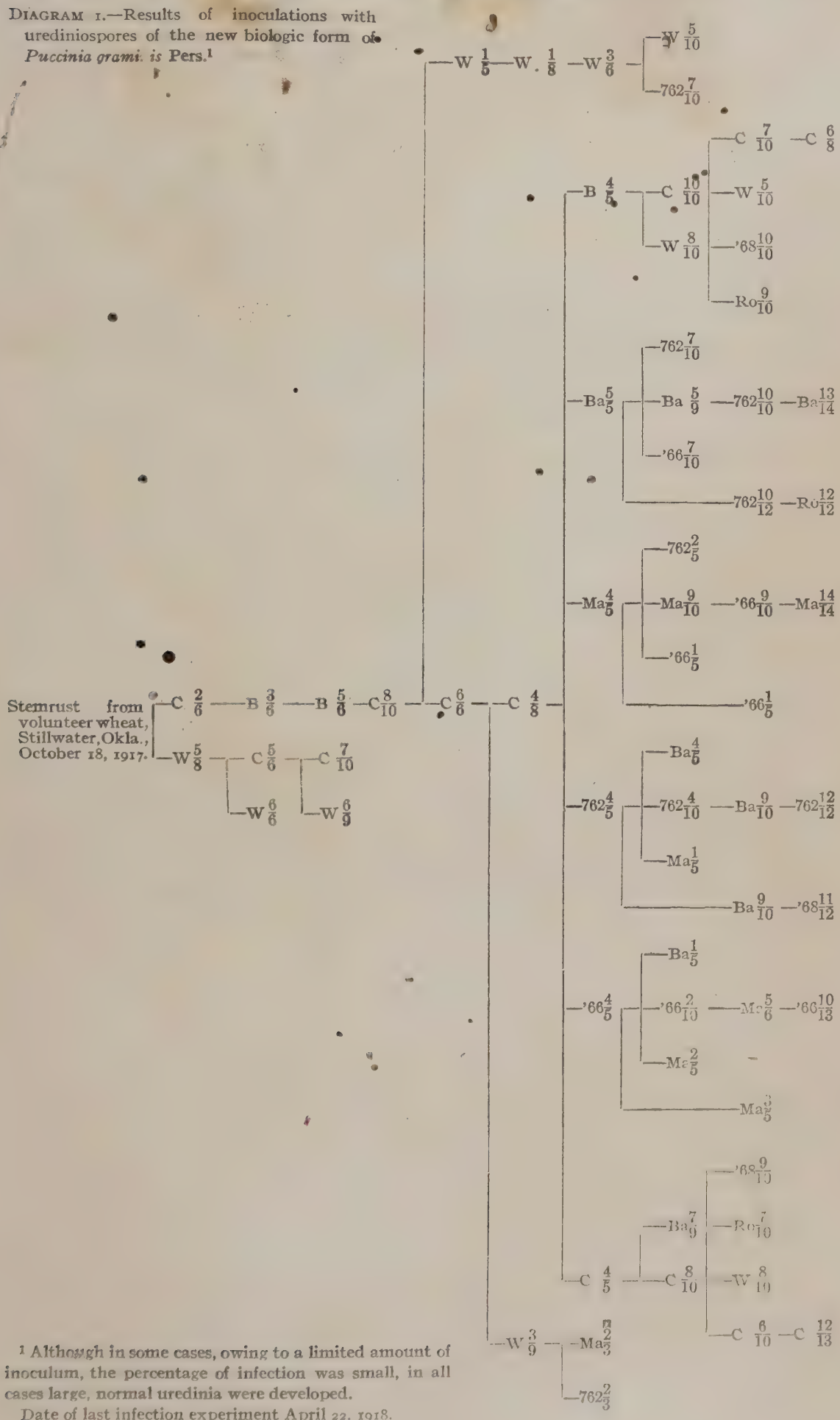
EXPLANATION OF DIAGRAM 1

In diagram 1 Haynes bluestem wheat is represented by W, club wheat by C, Kanred P762 by 762, P1066 by '66, P1068 by '68, Barletta by Ba, Marquis by Ma, Royalton by Ro, and barley by B. Transfers are indicated by dashes; thus, C—B—means that the rust was transferred from club wheat to barley and all other hosts indicated in the same vertical column, as Ba (Barletta), Ma (Marquis), 762 (Kanred P762), etc. The results of inoculations are represented in the form of a fraction, the denominator indicating the total number of leaves inoculated and the numerator the number which became infected.

[1] STAKMAN, E. C., and PIEMEISEL, F. J. OP. CIT.

[2] MELCHERS, L. E., and PARKER, J. H. THREE VARIETIES OF HARD RED WINTER WHEAT RESISTANT TO STEM RUST. *In* Phytopathology, v. 8, no. 2, p. 79. 1918.

DIAGRAM 1.—Results of inoculations with urediniospores of the new biologic form of *Puccinia grami. is* Pers.[1]



[1] Although in some cases, owing to a limited amount of inoculum, the percentage of infection was small, in all cases large, normal uredinia were developed.

Date of last infection experiment April 22, 1918.

TABLE I.—*Comparative results of inoculations with uredinospores of the three biologic forms of Puccinia graminis Pers. on six differential hosts*

Differential hosts.	*P. graminis tritici.*			*P. graminis tritici-compacti.*			The new biologic form.		
	Trials.	Result.	Degree of infection.	Trials.	Result.	Degree of infection.	Trials.	Result.	Degree of infection.
P762 (Kanred)	3	0/52	Immune.	2	17/18	Semiresistant.	9	58/77	Very susceptible.
P1066.....	2	0/23	...do.....	3	18/30	...do.....	7	34/58	Do.
P1068.....	3	0/32	...do.....	4	18/37	...do.....	3	30/32	Do.
Barletta..	4	35/37	Very susceptible.	17	23/174	Highly resistant.	8	53/69	Do.
Marquis...	2	17/18	...do.....	4	16/31	...do.....	8	40/53	Do.
Royalton .	1	8/9	...do.....	4	40/40	...do.....	3	28/32	Do.

Table I contains the summary of these inoculations, together with the results of inoculations with the *tritici* and *tritici-compacti* forms on the six differential hosts. It will be seen from this table that all of the varieties resistant to either *P. graminis tritici* or *P. graminis tritici-compacti* are highly susceptible to the new form.

From the above facts it is evident that the rust found in Oklahoma is neither *P. graminis tritici* nor *P. graminis tritici-compacti*. While only a few differential hosts have been tried, yet the action of the new rust on these hosts is so entirely different from that of the previously described biologic forms, that it must be considered as a distinct form, Extensive cross inoculations and an intensive morphological study are now under way.

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